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CARBOHYDRATE METABOLISM AND WINTER HARDINESS OF WHEAT[1]

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INTRODUCTION

Research conducted by many workers during the past half century has resulted in a body of evidence which indicates an important connection between soluble carbohydrates and the cold resistance of winter-hardy cereals. On the other hand, the frequent appearance of confusing and contradictory results and conclusions justifies additional research. Frequently cold resistance has been confused with winter hardiness; i. e., the resistance to frost injury at a specific time during hardening has received more attention than the development and maintenance of cold resistance throughout the winter period. The exact nature of the hardening process and the role of various factors in this process are of fundamental interest.

In the breeding of desirable varieties of winter cereals the incorporation of winter hardiness is usually a necessary precaution. Rapid and reliable methods for varietal characterization which may supplement the field and controlled freezing tests being conducted for this purpose are greatly to be desired. There is, then, a need for information regarding the exact relationship between carbohydrate metabolism and winter hardiness and also regarding the possibility of adapting such information to the differentiation of varieties on the basis of their winter hardiness. In the past such studies have been largely confined to field-grown material subject to the effect of adverse environmental conditions. Unquestionably this lack of control accounts for many of the contradictions found in the literature. An examination of the relationship between carbohydrate metabolism and winter hardiness of wheat as determined under controlled conditions has been undertaken with the results and conclusions as herewith reported.

LITERATURE REVIEW

No single source as yet provides an adequate review of the literature bearing on the relationship of soluble carbohydrates to winter hardiness of cereals. However, papers by Schaffnit (*30*),[2] Åkerman (*1*), Fuchs (*9*), and Levitt and Scarth (*17*) include rather extensive

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[2] Italic numbers in parentheses refer to Literature Cited, p. 24.

literature reviews, and the reader is referred to them for a more adequate treatment than space permits in the present paper.

The work of numerous investigators has established that coincident with a pronounced increase in cold resistance during the advent of the low temperatures of fall and winter, there is a pronounced increase of soluble carbohydrates in the wheat plant. A relationship has been established between low-temperature hardening, sugar accumulation, and radiation intensity. Among others, Tumanov (*36*, *37*), Mudra (*20*), Dexter (*5*), and Constantinescu (*4*) have demonstrated that light is necessary for the normal hardening of winter cereals and have likewise shown that the lack of hardening under light deficiency conditions is accompanied by a lack of sugar accumulation. Further emphasizing the importance of light in the development of cold resistance, Tumanov (*37*) and Suneson and Peltier (*34*) showed that any shading of the hardening plants, whether continuous or periodic, resulted in decreased cold resistance.

Attempts to express varietal differences in winter hardiness of cereals by carbohydrate analysis have not been entirely successful. Åkerman (*1*) was able to arrange 11 varieties of winter wheat in the order of their relative cold resistance by means of sugar and dry-matter determinations. Lebedincev et al. (*16*) experimented with 44 varieties of wheat, 16 of rye, and 4 of barley and showed that while individual variation in cold resistance was difficult to express by sugar analysis, the varieties could be arranged into hardy, medium-hardy, and nonhardy groups by this means. Mudra (*20*) was likewise able to establish a correlation between analysis and field-exhibited cold resistance. On the other hand, Newton (*21*, *22*) reported that dry matter and sugar increases, while associated with hardening, could not be used for varietal differentiation. Martin (*19*) and Salmon and Fleming (*27*) found a lack of correlation between cell sap solids and cold resistance of winter wheat. Timofejeva (*35*) and Balde (*2*) reported that there was no clear-cut agreement between sugar content and varietal differences in cold resistance. Govorov (*10*) found no correlation between dry-matter content and hardiness and no difference between winter and spring cereals in this respect. Janssen (*11*), in an experiment designed to study the effect of date of planting on winter hardiness of wheat, found that the plants of one of the planting dates which resulted in low cold resistance, contained the highest content of sugars. Further, Dexter (*8*) reported that an increase in sugars at low temperatures may or may not be accompanied by decidedly increased hardiness.

It is obvious from the above that more information must be obtained before any comprehensive picture of the relationship between winter hardiness and carbohydrate metabolism may be drawn.

MATERIALS AND METHODS

EXPERIMENTAL MATERIAL

Winter wheat (*Triticum aestivum* L.) was selected as a cereal grain readily adaptable to experimental procedure and one of which samples covering a wide range of known varietal winter hardiness are easily procurable. Table 1 shows the winter-hardiness characteristics, as outlined by Quisenberry (*25*), for the six varieties used throughout the present study. It may be seen from table 1 that the actual winter

survival in the uniform winter-hardiness nurseries of the Great Plains area during the period 1930–37 ranged from 71.5 percent for Minhardi to 44.7 percent for Fulcaster. In addition to the above varieties of winter wheat, a spring wheat, either Marquis or Ceres, and Tennessee winter barley were used on occasion for comparative purposes.

The seedling material used for hardening, dehardening, and freezing experiments was obtained by growing the grain in flats of fertile greenhouse soil in the greenhouse for a 3-week period following the date of planting. An attempt was made to hold the greenhouse temperature at approximately 67° to 70° F., but in the case of the early fall planting outside conditions caused a notable rise in the average growing temperature. Each flat contained all six varieties of winter wheat and in each series of six flats six different positions were occupied by each variety.

TABLE 1.—*Winter-hardiness classification of the six varieties of winter wheat used for controlled studies*

Variety	C. I.[1] No.	Description	Field survival 1930–37[2]	
			Weighted average	In ratio to Karkof
			Percent	*Percent*
Minhardi	5149	Soft red winter	71.5	123.3
Minturki	6155	Hard red winter	67.4	116.2
Cheyenne	8885	do	59.7	102.9
Kawvale	8180	Soft red winter	58.2	93.6
Blackhull	6251	Hard red winter	45.8	79.0
Fulcaster	6471	Soft red winter	44.7	77.1

[1] C. I. denotes accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry U. S. Department of Agriculture.
[2] Data for average field survival taken direct from published data of Quisenberry (*25*).

During the winter of 1937–38 three experiments were conducted, the same six varieties of winter wheat being used throughout. The material of experiment A was hardened through the period October 27 to December 10, 1937; experiment B through December 13, 1937, to February 8, 1938; and experiment C through February 10 to April 4, 1938. In this manner hardening was carried on under both decreasing and increasing day lengths. Experiments not reported in detail in this paper indicated that controlled hardening of the type used results in the maximum level of reserves being attained in about 50 days. For example, the average sucrose and dry-matter content for the crowns of two varieties of winter wheat were 15.99 and 30.22 percent, respectively, after 48 days of hardening as compared with 15.73 and 29.70 percent after 70 days. Accordingly, the experiments were terminated after about 7 to 8 weeks of hardening.

HARDENING, DEHARDENING, AND FREEZING

The control equipment for the experimental hardening and freezing of crop plants has been described by Peltier (*24*). In essentials the hardening room consists of a double-glassed greenhouse held by refrigeration at any constant temperature between freezing and normal growing temperatures. The freezing room may be held with little variation at any desired temperature from 0° to −30° C. Through-

out the work herein reported the hardening room temperature was maintained at approximately 2°.

The experimental plants were moved from the warm greenhouse to the hardening room at the 3-week seedling stage and held thereafter continuously at approximately 2° C., and were watered when necessary. As desired, samples were taken for analysis, flats were removed to the freezing chamber for survival tests, or were transferred back to the warm greenhouse for dehardening.

For purposes of this report the term "dehardening" means the process involved when a previously low-temperature hardened plant is caused to lose a degree of its cold resistance by the influence of the higher temperatures conducive to the transition from dormancy to active growth.

ANALYTICAL METHODS

Sampling

Analyses are reported for several somewhat different types of material. During the early part of the year (experiments A and B) samples referred to as "leaves" represented the section of the seedling above ground level, material other than leaves being only a small part of the whole. When both crown and leaf tissue were sampled, as in experiment C, the whole plant, after removal of the roots, was divided into two parts: (1) The leaf blades and (2) the remaining crown, stem, and leaf-sheath materials. This crown and stem tissue is referred to as "crown." In one instance—the dehardening phase of experiment B—the whole plant, after removal of the roots, was used for analysis.

No matter what plant section was selected, each sample represented a composite of from 30 to 50 plants, i. e., a composite containing from 5 to 8 plants from each of 6 flats. The selected material, after cleaning and removal of any dead tissue, was cut up finely with scissors, mixed thoroughly, and placed in an aluminum moisture can of appropriate size. All operations were conducted as rapidly and uniformly as possible. Sampling of hardened material was performed in the hardening room. Sampling of unhardened or dehardened material was done in the warm greenhouse but the sealed cans were placed in the hardening room as soon as obtained. Unless otherwise indicated, all sampling was conducted between 10 and 11 a. m.

Following the sampling, the material was kept under refrigeration except during the brief periods when it was being transported to the laboratory or being weighed. It was established that minor variations in this sampling procedure had essentially no influence on the subsequent dry matter and sugar determination.

Determination of Dry Matter

An appropriate amount (4 to 5 gm.) was rapidly weighed into a small aluminum moisture can and the cover tightly affixed. This can with its contents was placed on a ½-inch solid aluminum shelf in an oven held at 140° C. and left there for 15 minutes. By this means steam is produced almost immediately inside the can, thereby rapidly inactivating the enzymes without charring. Following this preheating treatment, the material was dried overnight under vacuum at 90°, cooled in a desiccator, reweighed, and the percent dry matter calcu-

lated. In addition, this method of heating and drying produces a dry sample which, if desired, may be quantitatively transferred to a flask and extracted for sugar determination.

Determination of Sugars

Either 4 to 5 gm. of the green material or the entire residue resulting from one of the dry-matter determinations was placed in a 100-cc. Kohlrausch volumetric flask. A few drops of toluol were added and the flask was covered with a watch glass and then gently heated to permit permeation of the plant material with toluol vapor. Fifty to sixty cubic centimeters of boiling water were then added and extraction carried out for 1 hour on the steam bath. Longer periods of extraction have not proved beneficial and under the above conditions neither has the addition of calcium carbonate for neutralization.

Following extraction, reducing sugars were determined by the ferricyanide method, as described by Blish and Sandstedt (*3*), using 0.10 N reagents as suggested by Sandstedt (*28*). To the cooled extract 4 cc. of 10-percent sulfuric acid and 4 cc. of 12-percent sodium tungstate solution were added, the volume brought up to 100 cc. with water, filtered, and a 5-cc. aliquot of the filtrate taken for reduction. It was realized that all of the nonsugar material which is capable of reducing ferricyanide under the conditions of the method is not removed by the clarifying agents used. A conventional copper-reduction method following alcohol extraction and lead acetate clarification was used as a standard and some 25 samples run by both this and the ferricyanide method. Assuming true values for reducing sugars by the copper-reduction method, it was possible to calculate the reducing-sugar content of the material from the volume of ferricyanide reduced. Reducing sugars are conveniently expressed as glucose.

Sucrose was determined by a slight modification of the method proposed by Sandstedt (*29*). A 5-cc. aliquot of the filtrate used above for reducing-sugar determination was placed in a test tube and heated in the boiling water bath for 7 minutes. This proved adequate to hydrolyze the sucrose. After cooling, the ferricyanide solution was added and reduction carried on as customary. Subtraction of the volume of ferricyanide reduced in the previous determination of reducing sugars from this final total volume of reduced ferricyanide gave the reduction attributable to inverted sucrose. The use of the sucrose-ferricyanide table of Sandstedt (*29*) permitted ready transposition into milligrams of sucrose. A comparison of this method with the conventional hydrolysis and copper-reduction procedure showed close agreement between the two.

Method of Recording Results

There has been considerable variation in the manner in which investigators have expressed the sugar content of plant material. Sugar content has been reported as percent of dry weight, percent of green weight, weight per 100 plants, and as percent in the water actually present in the fresh plant tissue. It is obvious that if the sugar content of the plant is considered significant by reason of its osmotic effect its role as a solute must be the important factor. Whether or not osmotic relationships are of prime significance in cold

resistance there is no apparent disadvantage in using this method of expression. Accordingly, sugar is recorded as the percent glucose, or sucrose, in the total water actually present in the fresh, green seedling material at the time of analysis.

EXPERIMENTAL RESULTS

THE HARDENING PROCESS

General Considerations

To be comparable to natural field hardening, any method of artificial low-temperature hardening should produce comparable plants

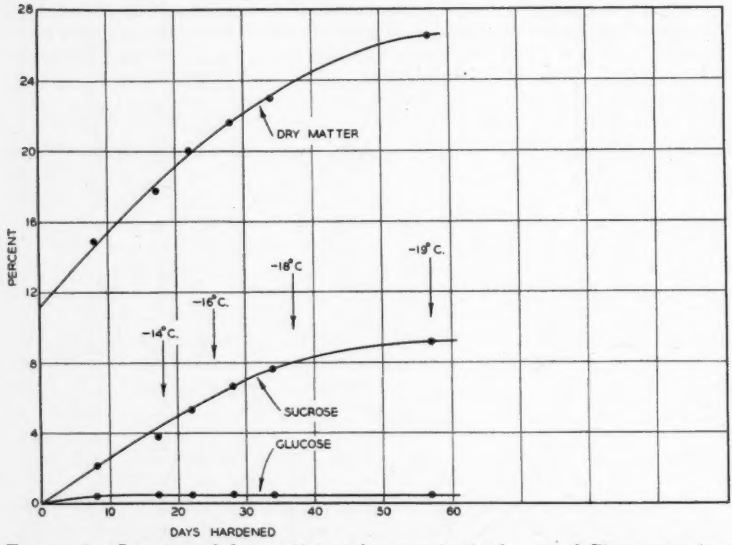

FIGURE 1.—Increase of dry matter and sugars in the leaves of Cheyenne wheat during the 8 weeks of controlled hardening. Inserted temperatures are those at which a 24-hour exposure resulted in 20 percent of the plants being killed.

both as regards physiological and morphological make-up. Suneson and Peltier (*32*) have indicated that with wheat seedlings under controlled low-temperature hardening (29° to 35° F.) the maximum degree of cold resistance is reached in 3 weeks. They likewise found (*34*) that after this period of hardening the cold resistance of the plants, as measured by freezing tests, was much less than that of field-hardened material. Their data indicate effective freezing temperatures to be of the order of −11° C. for the control-hardened as compared to −20° to −24° for the field-hardened material. Further suggesting that control and field-grown plants were not strictly comparable is the observation of these authors (*32*) that during 3 weeks of hardening the decumbent stature of the seedlings increased only "somewhat."

The present work does not support the above conclusions.

Figure 1 shows the physiological changes taking place in the leaves of one representative variety of winter wheat during a prolonged period of controlled low-temperature hardening. Temperatures

necessary to kill approximately 20 percent of the plants under a controlled 24-hour freeze are indicated at various stages of the hardening period. It is apparent from figure 1 that at least 7 weeks of continuous low-temperature (2° C.) hardening were necessary before the maximum hardiness was attained. Dry-matter and sucrose content increased throughout this period as did the resistance to freezing. Reducing sugars as glucose reached a maximum during the first 2 to 3 weeks of hardening and showed little quantitative change from then on. The actual increase in cold resistance appears to be correlated best with changes in dry-matter and sucrose content.

The changes taking place under artificial hardening seem closely comparable to changes which may be observed under natural field conditions. When the seedlings are placed in the hardening room growth is greatly retarded. The leaves gradually become "crisp" and assume a darker green color. Growth habit becomes progressively more decumbent as the peak of hardiness is approached until the plants finally assume a type of growth practically indistinguishable from that of field-hardened material.

Figure 2 illustrates the decumbent habit of artificially hardened plants. The two plants at *A* are typical of well-hardened seedlings. For contrast, the plant at *B* was selected from similar plants which had passed through their dormancy and started active "spring" growth. Their roots and all dead material were removed from the plants illustrated in figure 2 before they were photographed.

Artificially hardened wheat plants closely resemble field material in physiological make-up and actual cold resistance. This was established by comparing crown analyses on comparable wheat plants both field and artificially hardened and both at their approximate maximum cold resistance. The field material contained 26.65 percent of dry matter and 9.11 percent of sucrose; the artificially hardened, 26.55 percent of dry matter and 11.57 percent of sucrose. In both cases the temperature necessary to kill 20 percent of the plants was of the same order, i. e., within a degree of −20° C. From the foregoing it appears evident that artificial hardening is to a high degree comparable to the processes involved in natural field hardening.

Light and Hardening

Several authors have established that light is necessary for the normal hardening of winter wheat plants. In addition, Dexter (*5*) has demonstrated that under conditions of constant low-temperature hardening of wheat greater cold resistance develops under a long than under a short day. Controlled hardening technic is well adapted to studies of this nature.

In the early fall three series of flats, each flat containing the same six varieties of winter wheat, were placed in the hardening room and held at three different levels of light intensity for 43 days. The different levels of light intensity were obtained by shading, and consisted of unshaded or "full light," slightly shaded or "intermediate light," and heavily shaded or "low light." At the end of the 43-day period of hardening several flats from each series were frozen for 24 hours at −15° C. and the survival counted at the end of a 2-week recovery period. The average survival of the six varieties was 84 percent for the "full light," 64 percent for the "intermediate light," and 11 percent for the "low light" series.

Samples of the leaves of the Minturki and Blackhull varieties from unfrozen flats under "full" and "intermediate" light were analyzed

FIGURE 2.—Decumbent habit of artificially hardened winter wheat plants (*A*) compared to the erect habit of a plant in active growth (*B*).

for dry matter and sugars. The leaves of the plants under "low light" were so yellow and necrotic as to make their use for analysis inadvisable. Table 2 shows the results obtained. It is obvious that the

decreased ability to develop cold resistance under lowered light intensity is accompanied by a decreased elaboration of dry matter and sugars.

TABLE 2.—*The effect of two levels of light intensity during low-temperature hardening of wheat on the accumulation of dry matter and sugars*

Variety	Dry-matter and sugar content under—					
	Full light			Intermediate light		
	Dry matter	Sucrose	Glucose	Dry matter	Sucrose	Glucose
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Minturki	20.28	3.15	0.46	17.40	1.17	0.26
Blackhull	18.95	2.13	.39	16.23	0.54	.14

Since radiation is important in the development of cold resistance, a diurnal variation in cold resistance would be anticipated; i. e., cold resistance should reach its peak in the afternoon, drop somewhat during the following night, and then climb again during the succeeding day. Martin (*18*) and Salmon (*26*) reported that they found a decided tendency for hardening cereal plants to be injured more by day freezing than by night freezing. The diurnal variation in carbohydrate reserve is illustrated in table 3. The data reported are the averages of crown analyses for three varieties of hardening winter wheat plants grown in soil in 1939 and for two series grown and hardened as sand cultures in 1940. These data show that an increase in dry-matter and sugar content during the day is followed by a decrease during the night. It is significant that the decrease is only to a point considerably above that of the morning before. The hardening process may then be expressed as a zigzag progression, the cold resistance on each successive morning, though not equal to the highest point reached the day before, being an effective increase over that of the morning before. This of course assumes optimum hardening conditions.

TABLE 3.—*Diurnal variation in the dry-matter and sucrose content of hardening winter wheat seedlings*

Culture medium and sampling time	Dry matter	Sucrose
	Percent	*Percent*
Soil culture:		
Mar. 23, 1939—9:00 a. m.	25.17	8.55
Mar. 23, 1939—5:00 p. m.	26.73	10.55
Mar. 24, 1939—9:00 a. m.	26.57	9.94
Sand culture:		
Mar. 15, 1940—9:00 a. m.	23.10	8.94
Mar. 15, 1940—5:00 p. m.	24.70	10.92
Mar. 16, 1940—9:00 a. m.	24.15	10.45
Mar. 16, 1940—5:00 p. m.	25.65	11.66
Mar. 17, 1940—9:00 a. m.	24.74	11.01

As regards the influence of varied length of day during continuous low-temperature hardening there was no pronounced effect. So far as could be determined hardening progressed with about the same effectiveness either under the increasing day lengths of late winter

and early spring or under the decreasing day lengths of fall and early winter. The same morphological and carbohydrate changes took place during either period, resulting in the development of approximately the same degree of cold resistance.

THE DEHARDENING PROCESS

It has been indicated above that by dehardening is meant the process whereby hardened winter-wheat seedlings are caused to lose a substantial part of their cold resistance by transferring them from low

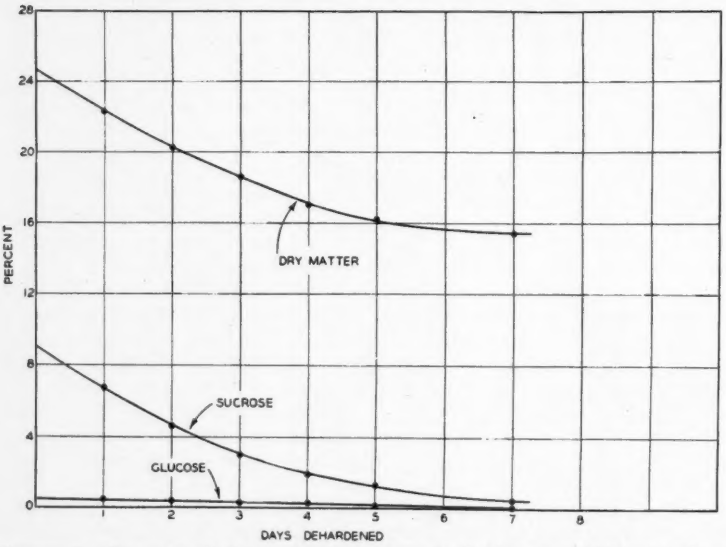

FIGURE 3.—Decrease of dry matter and sugars in the leaves of Minturki wheat during 7 days of high-temperature dehardening.

temperatures to the higher-temperature conditions optimum for active growth. Several investigators have demonstrated this loss of cold resistance under the influence of temperatures high enough to stimulate activity. Laude (*14*, *15*) in particular has made an extensive study of the transition of winter cereals from dormancy to active growth. He concluded that transition as effected under favorable greenhouse conditions was essentially comparable to winter-spring transition in the field. Coincident with a progressive loss of cold resistance, he found an increase in water content and in the amount of expressed leaf juice, and a decrease in the total solid content of the sap. He likewise postulated resistance to loss of hardiness as a varietal characteristic.

Figure 3 shows typical curves illustrating the changes in dry-matter and sugar content when hardened winter wheat plants are placed under temperature and light conditions conducive to active growth. In this instance the analysis was on the "whole plant," only the roots and dead material being discarded. The plants had previously been held under controlled low-temperature hardening for 34 days and had

reached the level of physiologic hardiness indicated in figure 3 by zero days dehardening.

When the curves of figure 3 are compared to those of figure 1 it is obvious that there is a striking similarity between the two processes. Hardening is accompanied by an increase, dehardening by a comparable decrease, in dry-matter and sugar content. In other words, dehardening under these conditions may be described as a rapid reversal of the low-temperature hardening process.

ROLE OF LEAVES, CROWNS, AND ROOTS IN COLD RESISTANCE

Before any attempt is made to correlate composition with cold resistance, localization of the plant section responsible for survival is essential. Most investigators who have attempted to correlate chemical composition of winter cereals with cold resistance have used the leaves as analytical material. In fact, Newton and Anderson (*23*) stated that "the leaves are the organs which determine winter survival in wheat." The importance of the leaves in winter hardiness is emphasized by the defoliation experiments of Dexter (*6*) and Suneson and Peltier (*33*), who demonstrated that either artificial or natural defoliation interferes with the normal hardening process in winter wheat. It is only natural that leaf survival and hardening should be interrelated since the development of cold resistance apparently is associated with photosynthetic processes.

When, however, it is desired to compare varieties of winter wheat as regards cold resistance at any specific time during their hardening period, attention must be directed to this particular factor rather than to the general question of ability to harden. Martin (*19*) and Van Doren (*38*) found that the leaves of hardened winter wheat plants were less cold resistant than the crowns. Van Doren (*38*) likewise found a direct correlation between the bound water content of the crowns and cold resistance, this relation being just reversed for the leaves. Janssen (*11*) and Dexter (*7*) considered the crowns to be the seat of cold resistance and confined their analyses to this section of the winter wheat plant. Moreover, the work of Janssen (*12*) would suggest that the crown is the only organ involved in survival of hardened winter wheat plants. He removed both leaf blades and roots from field-grown plants during February and March and compared the development from the remaining crowns with the development of comparable untouched plants. He found some differences in growth but no decrease in final yield as a result of this drastic treatment.

In this investigation, likewise, it has been observed that the leaves have little if any role in the ability of hardened winter wheat seedlings to survive low temperatures. For instance, many comparisons in frost resistance were made between hardened plants which had been stripped of all their leaf blades immediately before freezing and duplicate undefoliated plants. No distinguishable difference was found in survival after the conventional 2-week recovery period. In all cases, even with 100-percent survival, the action of the low temperatures resulted in complete defoliation of the check plants, thus placing both sets on a comparable basis.

A more specific illustration was provided by the results of an experiment designed for another purpose. In a differential nitrogen nutrition experiment wheat was grown in sand culture, one part

receiving only ammonia nitrogen, the other exclusively nitrate nitrogen. After 3 weeks of growth the two series were hardened for 11 days, frozen for 24 hours at −15° C., and then removed to the warm greenhouse for recovery. The day following the freeze the plants of the nitrate series appeared to be badly damaged with all the leaf growth completely destroyed. On the other hand, the leaves of the plants in the ammonia series were green, crisp, and showed little injury. This condition persisted for several days after which period the leaves of the plants supplied only ammonia nitrogen turned yellow and killed back badly. About this time new leaves were beginning to arise from the crowns of the plants in the nitrate series. When the plants were examined for survival at the end of 2 weeks 75 percent of the plants of the nitrate series showed active new growth as compared to 11 percent for those of the ammonia series. This difference in behavior could not be attributed to root survival, as in this instance the plants of both series showed little root injury. If anything the plants of the ammonia series showed slightly less root injury than those of the nitrate series. On more detailed examination it was found that lack of growth was directly associated with the severity of crown injury.

Analytical data on crowns and leaves of different varieties of hardened winter wheat show that cold resistance is more closely correlated with crown composition than with leaf composition. This becomes very striking in some instances. An example is shown in table 4, where two varieties of soft red winter wheat are compared as to relative cold resistance of hardened seedlings and composition of comparable material. The dry-matter and sugar content of Kawvale crowns is considerably higher than that of Fulcaster crowns, thus showing agreement with relative cold resistance. On the other hand, the position is just reversed for the leaves: the dry matter and sugar content of these organs being higher in the less cold-resistant plants.

TABLE 4.—*Comparison of crown and leaf composition of two varieties of winter wheat with their relative cold resistance*

Variety	Freezing survival	Crown composition			Leaf composition		
		Dry matter	Sucrose	Glucose	Dry matter	Sucrose	Glucose
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Kawvale	100	20.50	6.18	0.72	21.70	2.24	0.31
Fulcaster	67	19.10	4.96	.62	21.98	2.69	.34

The roots of winter cereals have received very little attention in considerations of cold resistance. Zacharowa (*39*) attempted to correlate root composition and cold resistance of rye plants. Lamb (*13*) made an extensive study of the varietal characteristics of wheat roots as related to winter injury, particularly by soil heaving. Suneson and Peltier (*31*) noted that the roots of field-hardened winter wheat plants were less cold resistant than the crowns. This was confirmed by the present authors for soil-grown, artificially hardened plants. In addition, the problem was further clarified by the results of differential nutrition experiments with sand cultures.

Many thousands of sand-culture-grown wheat plants have been hardened, frozen, and subsequently examined in detail for root, crown, and leaf injury. No correlation whatever has been found between root survival and plant survival. Many instances were noted in which complete killing of the roots was coincident with 100-percent survival of the plants associated with these roots. On the other hand, under certain nutritional treatments instances were noted where the tops of plants showed 100-percent killing in spite of excellent root survival.

The role of differential nutrition in cold resistance will not be considered in this paper. For the present purpose one series illustrating the relationship between root injury and plant survival is presented without any consideration of the nutritional significance. The data are presented in table 5. The different types of nutrition are represented only by letters. Plant survival is expressed as exact percent and root survival as six different levels from zero to 100 percent. The only conclusion permitted by the data is that, under these conditions, root injury is of no consequence in the cold resistance of the plants.

TABLE 5.—*Comparison of plant survival with root injury for winter wheat grown and hardened under 11 different nutrient conditions indicated by letter*

Nutrition	Plant survival	Root survival [1]	Nutrition	Plant survival	Root survival [1]
	Percent	*Percent*		*Percent*	*Percent*
A	81	0	G	59	20
B	81	80	H	55	100
C	77	40	I	38	20
D	71	60	J	38	60
E	69	60	K	16	80
F	59	60			

[1] "Root survival" is based on observed frost injury, 100-percent survival indicating no injury and 0 indicating complete killing. Root injury was estimated on the basis of the degree of discoloration and tissue break-down when the plants were examined for survival 2 weeks after freezing.

The above data suggest that the cold resistance of wheat roots is of slight consequence in the survival of the frozen wheat plant. Accordingly, little attention has been directed towards the determination of the carbohydrate reserves of these organs. For incidental information a few analyses were made on the roots of hardened wheat plants. The average dry-matter and sucrose content for the roots of the plants in five series under differential nutrition was 15.05 percent and 3.75 percent, respectively. Analyses of the crowns of the same plants showed the dry-matter and sucrose content of these organs to be 28.57 percent and 12.42 percent, respectively.

VARIETAL DIFFERENTIATION

The development and maintenance of cold resistance, while not the only factor governing the winter hardiness of wheat, is in many areas the chief one. Even when considering this one factor of low-temperature hardening several modifying factors must be taken into account. The relative winter hardiness of a particular variety will depend not only on the maximum cold resistance which it can attain but also on the speed with which it can reach this level and its resistance to dehardening influences. The importance of the leaves in the hardening process should not be underestimated. It is their

function to carry on the metabolic processes leading to the development of cold resistance in the crown tissue. It is, then, essential that the leaves develop sufficient cold resistance so that their survival may permit the fulfillment of this function.

Figures 4, 5, 6, and 7 show graphically certain composition changes taking place in the six previously described varieties of winter wheat when subjected to uniform hardening and dehardening influences. In all cases both the glucose and sucrose content are expressed as percents of the water present in the plant tissue. The data will be considered independently as experiments A (fig. 4), B (fig. 5), and C (figs. 6 and 7), and then in relation to each other.

The material of experiment A was grown in the usual manner in the warm greenhouse and then transferred to controlled low-temperature hardening conditions on October 27, 1937. Several flats of Tennessee winter barley and of Marquis spring wheat were included for comparison. As a consequence of unseasonably warm weather during the early growth stage, it proved impossible with this one series to maintain temperatures as low as the desired 67° to 70° F. This resulted in a rather rank growth and necessitated a 4-week growth period before the desired uniformly tillered seedlings were obtained. Consequently when subjected to hardening the seedlings were a week older, larger, and more erect than those of experiments B and C.

After 30 days of controlled hardening, several flats of the material in experiment A were frozen at −15° C. for 24 hours and survival determined after a recovery period of 2 weeks in the warm greenhouse. The percentage survival for the six winter wheat varieties was Minhardi 86 percent, Minturki 90, Cheyenne 78, Kawvale 74, Blackhull 61, and Fulcaster 59 percent. These data show fair agreement with the field response as given in table 1 and indicate that the varietal response to controlled hardening approximates average field survival. Winter barley and spring wheat, hardened and frozen in an identical manner with the above treatment, showed zero survival.

Analyses were made on the leaves at intervals during a 44-day hardening period. As may be seen from figure 4, the behavior of this material was quite erratic. However, certain general trends may be established. Dry-matter content increased regularly and rapidly during the first 10 to 20 days of hardening, after which time the rate of increase was somewhat less pronounced. Reducing sugar content had a similar trend. The curve for sucrose content shows very little increase during the first 9 days, a very rapid increase during the next few days, and a somewhat slower rate of increase during the remainder of the hardening period. As far as varietal trends are concerned the correlation with known cold resistance or with field-exhibited winter hardiness is poor except that the dry-matter and sugar curves for the three most cold-resistant varieties are considerably higher than for the three varieties of lesser cold resistance. Freezing data showed separation into three groups: Minhardi and Minturki, high; Cheyenne and Kawvale, intermediate; and Blackhull and Fulcaster low. The pronounced lack of ability of the winter barley and spring wheat to attain any high level of reserves agreed well with observed freezing reaction.

After 49 days of hardening a number of the flats of experiment A, similar to the flats used to obtain hardening data, were removed to the

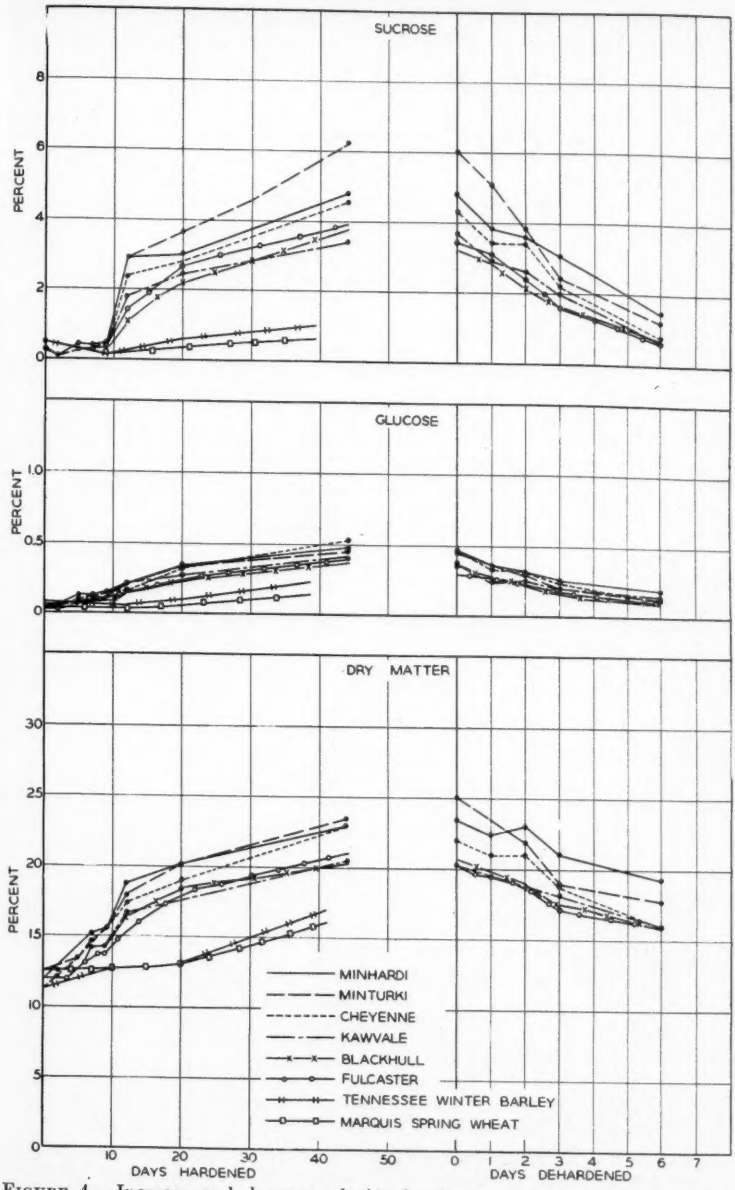

FIGURE 4.—Increase and decrease, during hardening and dehardening, of dry matter and sugars in the leaves of six varieties of winter wheat hardened during the period October 27 to December 10, 1937. Samples taken after 0, 2, 5, 7, 9, 12, 20, and 44 days of hardening and after 0, 1, 2, 3, and 6 days of dehardening. Experiment A. (Tennessee winter barley and Marquis spring wheat are shown for comparison.)

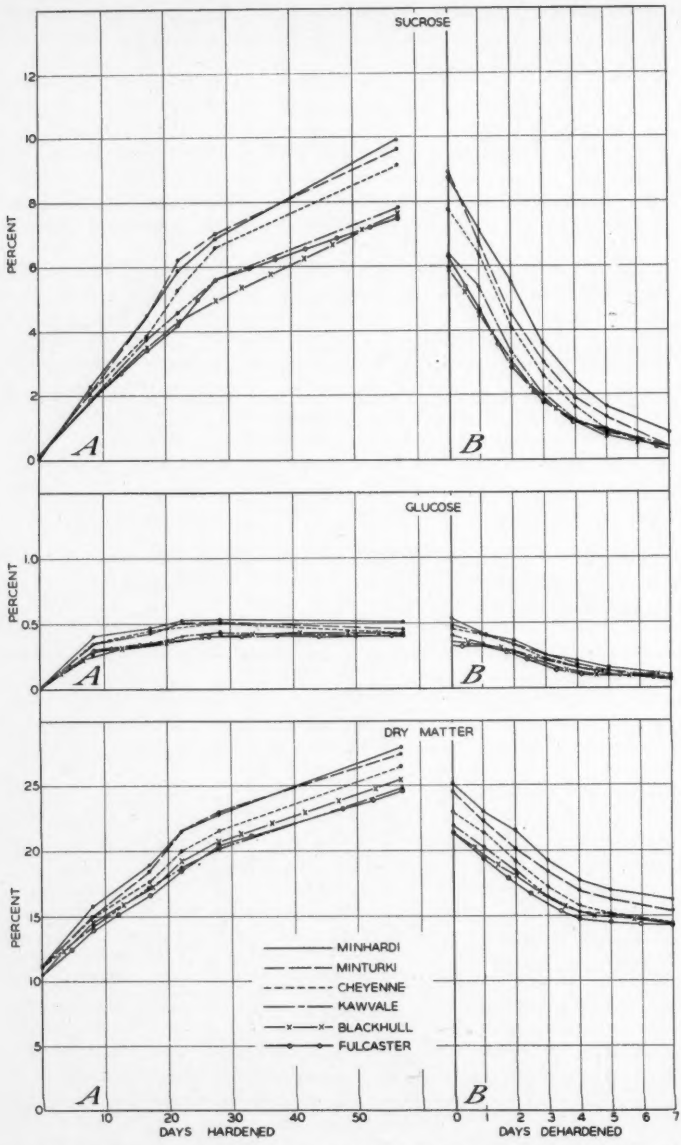

FIGURE 5.—Increase and decrease, during hardening and dehardening, of dry matter and sugars for the leaves (*A*) and whole plants (*B*) of six varieties of winter wheat hardened during the period December 13, 1937, to February 8, 1938. Samples taken after 0, 8, 17, 22, 28, and 57 days of hardening, and after 0, 1, 2, 3, 4, 5, and 7 days of dehardening. Experiment B.

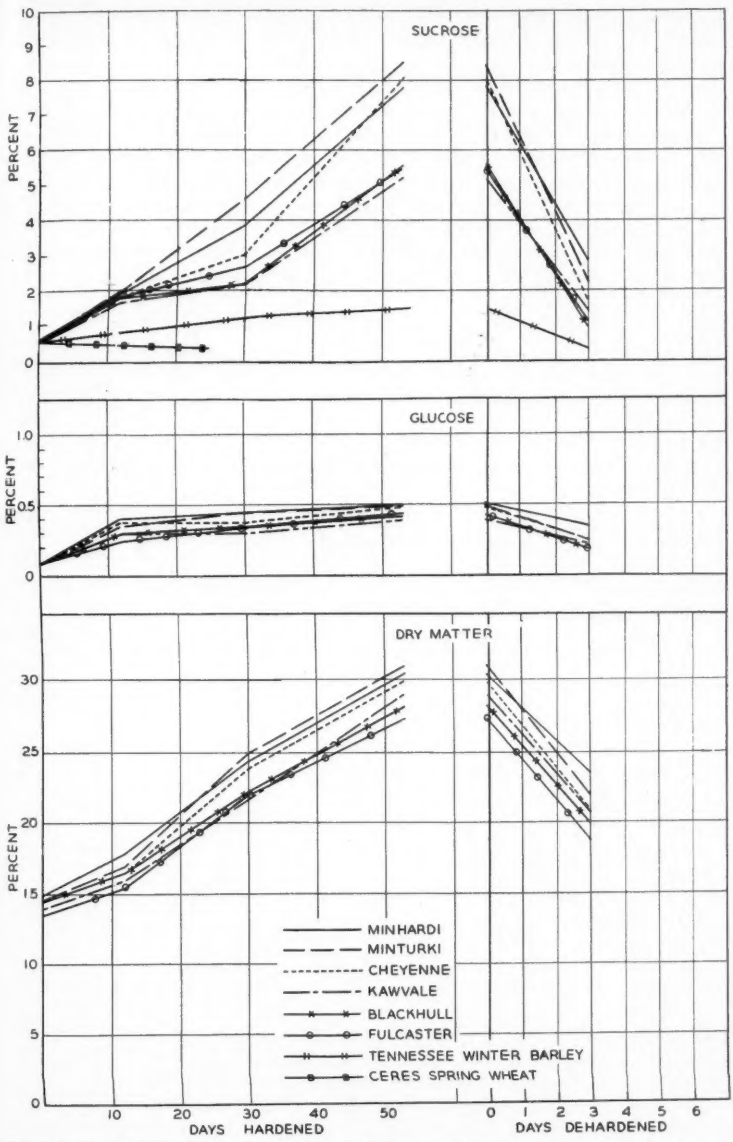

FIGURE 6.—Increase and decrease, during hardening and dehardening, of dry matter and sugars in the leaf blades of six varieties of winter wheat hardened during the period February 10 to April 4, 1938. Samples taken after 0, 12, 30, and 53 days of hardening, and after 0 and 3 days of dehardening. Experiment C. (Tennessee winter barley and Ceres spring wheat are shown for Comparison).

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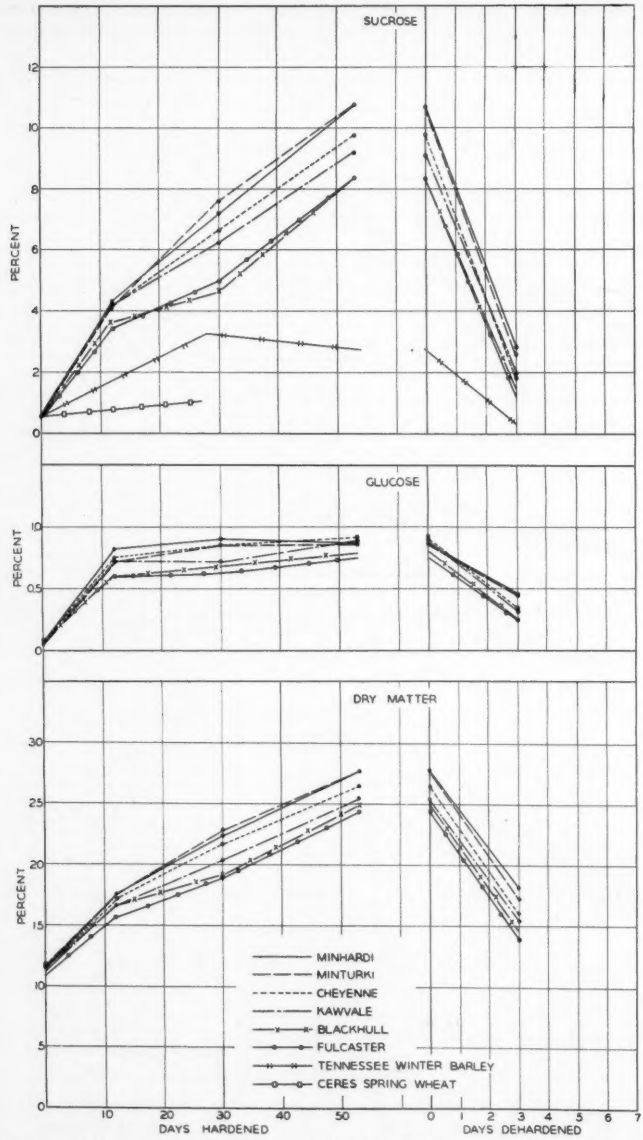

FIGURE 7.—Increase and decrease during hardening and dehardening of dry matter and sugars in the crowns of six varieties of winter wheat hardened during the period February 10 to April 4, 1938. Samples taken after 0, 12, 30, and 53 days of hardening, and after 0 and 3 days of dehardening. Experiment C. (Tennessee winter barley and Ceres spring wheat are shown for comparison.)

69° F. greenhouse and leaf analyses made at intervals during a 6-day dehardening period. As with the hardening plants, the nature of the growth resulted in unavoidable sampling errors and erratic results. However, definite and significant trends may be seen from figure 4. There is the usual progressive loss of reserves. However, the leaves of Minhardi and Kawvale resisted this loss to a pronounced degree. The net result is that the effect of dehardening was to orient the varieties in approximately the same manner as is observed in field behavior. Even after 6 days of dehardening the two most winter-hardy varieties retained their reserves to a relatively high degree.

Experiment B was carried out with the same six varieties of winter wheat as previously described. The seed was planted November 22, 1937, and grown for three weeks at 67° to 70° F. At the end of this period the seedlings were in good condition for hardening and analysis, i. e., actively tillered and exhibiting a uniform slightly decumbent growth habit. The flats were then removed to the hardening room and leaf samples taken for analysis at intervals during a 57-day hardening period.

As a check on the relative cold resistance of the wheat varieties several flats of material in experiment B were frozen after 37 days of hardening. A freezing treatment of −17.5° C. for 24 hours followed by a 2-week recovery period resulted in the following percentage survivals: Minhardi 62, Minturki 62, Cheyenne 50, Kawvale 47, Blackhull 13, and Fulcaster 12 percent. These data confirm those found for the material of experiment A inasmuch as the relative cold resistance of the varieties during controlled hardening is closely comparable to known average winter-hardiness response.

Data for dry matter, glucose, and sucrose in the plants of experiment B are presented in figure 5. The leaves of these plants showed a progressive accumulation of dry matter and sucrose throughout the whole hardening period. On the other hand, reducing sugars accumulated rapidly during the first few days, increased slowly up to about 4 weeks of hardening and then remained fairly constant for the remainder of the 8 weeks. The hardening curves show that some degree of varietal orientation is apparent almost from the initiation of hardening, this orientation becoming more pronounced with time. There is again the tendency for the varieties to segregate into two groups instead of the three groups indicated by freezing experiments. This is the result of the leaves of the Kawvale variety having about the same content of sucrose and dry matter as those of Fulcaster. As pointed out in table 4, the leaf composition of these two varieties tends to be similar, regardless of the higher degree of cold resistance exhibited by the Kawvale plants.

After 34 days of hardening a number of flats were removed to the 69° F. greenhouse and sampled at intervals over a 7-day dehardening period. The whole plant, less only the roots, was used for analysis. The data are recorded in the dehardening curves of figure 5. These curves show the same smoothness as those for hardening. Two or three days of dehardening resulted in the analyses showing a close correlation with winter-hardiness response. In fact at the end of 2 days of dehardening the varieties lined up in the exact order of known field response. Minhardi, and to some extent Minturki, leaves showed resistance to dehardening.

Experiment C was carried out with the same six varieties of winter wheat as used for the previous experiments. Three weeks after the planting date of January 20, 1938, the flats were removed to the hardening room and hardened in the customary manner for 53 days. Samples were taken at 0, 12, 30, and 53 days of hardening. After 53 days of hardening several flats were dehardened for 3 days, sampling being done at the end of this period. All samples were divided into two fractions for analysis, namely, the leaf blades and the crowns, and analysis carried out on each fraction independently. Results for dry matter, glucose, and sucrose are recorded for the leaf blades in figure 6 and for the crowns in figure 7. The sucrose content of Tennessee winter barley and of Ceres spring wheat were included for comparison. Because of shortage of material no data on freezing survival was obtained for the hardening plants of experiment C.

The data of figure 6 again show a progressive increase in dry matter and sucrose over the whole hardening period, this increase being somewhat more marked in the later stages of hardening. The curves for glucose again show an initial accumulation followed by very little further increase. The tendency for the usual orientation into two groups is marked. Three days of dehardening resulted in a good agreement of analytical and winter-hardiness data, with Minhardi leaves showing the usual resistance to dehardening. As in experiment A, the leaves of both the winter barley and the spring wheat showed a pronounced lack of ability to accumulate sucrose.

The curves in figure 7 show a pronounced and progressive increase in dry matter and sucrose content in the crowns throughout the entire hardening period. Those for glucose show the same type of rapid initial increase followed by little further accumulation as observed for the leaves. Orientation into the three groups established by freezing experiments is apparent for sucrose content at 30 days of hardening and is maintained at 53 days of hardening. The curves for dry-matter content show fair correlation with cold resistance, especially at 53 days of hardening. Three days of dehardening changes the relative position only slightly but what change there is tends to give a higher degree of correlation between composition and winter-hardiness response, especially as regards dry-matter content. As with the leaves, the crowns of the barley and spring wheat seedlings showed little ability to accumulate sucrose. As might be expected, the winter barley was intermediate between the spring and winter wheat in this respect.

Comparing the leaf-blade and crown analyses for experiment C (figs. 6 and 7), it is obvious that the dry matter and content the sucrose content of the crowns are correlated to a greater degree with varietal field winter hardiness than are those of the leaf blades. In addition, the relative crown composition is in excellent agreement with the freezing response shown by these artificially hardened plants in experiments A and B. In this particular experiment the leaf blades exhibited a higher dry-matter content than the crowns throughout hardening and dehardening. On the other hand, the sugar content of the crowns was consistently higher than that of the corresponding leaf blades, sucrose alone reaching, in the hardiest varieties, a concentration of over 10 percent calculated on the water basis, or over 25 percent of the dry matter present.

Direct comparision of the three hardening periods represented by experiments A, B, and C can be made only with respect to leaf composition. However, the results of experiment C suggest that the behavior of the leaves gives a fair indication of the magnitude of change taking place in the crowns. It is apparent from the data for the three experiments that the greatest accumulation of reserves occurred in the leaves of those plants hardened through the latter part of December, January, and the first part of February (experiment B). Very close behind were those plants hardened through February and March (experiment C). This difference seems to be the result of the more rapid hardening of the B plants during the first 30 days. In both cases the reducing-sugar content shows a similar response to low temperatures and cannot be considered of great significance in the general hardening picture with the possible exception of the early stage.

The plants showing the least ability to accumulate reserves were those hardened during November and the first part of December (experiment A). The lack of hardening ability, as well as the erratic results shown by this series, may be in part attributable to the nature of growth. The fact that the plants of experiment A were older, larger, and not possessed of the same desirable winter habit as those of the other two series makes the results of somewhat questionable significance. However, they do indicate the type of growth which is undesirable for controlled hardening and suggest that the hardening process is modified by the plant's growth habit. Under the constant low-temperature hardening conditions the nature of the response of the winter wheat plants to hardening under decreasing day lengths (experiment A) is little different from that under the influence of increasing day lengths (experiments B and C).

Crown analyses were made on the material of experiment B at 57 days of hardening only. At this stage of hardening the six varieties showed a range of from 27.65 to 24.43 percent dry matter, from 12.29 to 9.77 percent sucrose, and from 1.00 to 0.83 percent reducing sugars as glucose. Comparing these data with those of the experiment C crowns as recorded in figure 7, it is apparent that the plants hardened during late winter showed a somewhat greater accumulation of reserves than did those hardened during early spring. It is questionable whether or not this difference is sufficient to indicate any greater efficiency of hardening during the somewhat shorter days prevailing when the plants of experiment B were grown and hardened.

The data of experiments A, B, and C indicate that Minhardi plants show resistance to loss of reserves when the plants are subjected to dehardening conditions, this being particularly true for the leaves. That this is likewise true for cold resistance was demonstrated by freezing a number of flats of dehardening plants. The material used was part of experiment C and was that remaining when this experiment was terminated. At this time, early April, high outside temperatures made impractical the maintenance of sustained low temperatures in the hardening room. During the succeeding 3 weeks refrigeration was so manipulated that day temperatures were maintained at 65° F., and night temperatures gradually increased until at the end of this period they, too, approximated 65° F. Several flats of material were frozen after 10, 16, and 23 days of this dehardening treatment. A constant temperature and period of freezing was used, namely, −17° C. for 24 hours. The results are given in table 6.

TABLE 6.—*Cold resistance measured by survival of 6 winter wheat varieties, frozen at −17° C. for 24 hours, after 10, 16, and 23 days of slow dehardening*

Days dehardened	Survival of the 6 varieties					
	Minhardi	Minturki	Cheyenne	Kawvale	Blackhull	Fulcaster
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
10	97	95	95	92	25	17
16	92	72	57	65	3	13
23	60	9	3	8	0	0

The data of table 6 show that the Minhardi variety has pronounced resistance to dehardening influences. Even after 23 days of dehardening, when Blackhull and Fulcaster were completely killed and Minturki, Cheyenne, and Kawvale showed less than 10-percent survival, 60 percent of the Minhardi plants were in active growth after a 2-week recovery period. The results clearly indicate that the resistance which the Minhardi variety shows to loss of reserves during dehardening coincides with an ability to retain cold resistance.

DISCUSSION AND CONCLUSIONS

Climatic conditions vary widely in different regions of the winter wheat growing area of the United States. Considering winter hardiness only from the cold-resistance aspect, it is obvious that the requirements for a variety to be winter hardy in a southerly latitude are not necessarily the same as those permitting winter survival in a region farther north. For example, let us postulate a theoretical "winter" climate where early fall frosts are to be anticipated, where extremely low winter temperatures are exceptional, and where frequent warm periods alternate with cold throughout the winter. A variety with favorable winter hardiness for this specific climate should rapidly attain a degree of cold resistance in the fall, and while a high level of cold resistance is not necessary at any period, resistance to the dehardening influence of high temperatures is essential. Modification of the climatic conditions postulated above necessitates modification of the winter-hardiness characteristics which would qualify a variety as ideally adapted. The difficulty of observing such varietal characteristics in the field suggested the present studies in which the reaction to controlled hardening and dehardening conditions may be determined.

When winter wheat plants were grown with uniform soil conditions and hardened under exactly comparable conditions of low temperature and light, increases in dry matter and sucrose content were coincident with increases in cold resistance. Winter wheat varieties which showed a high level of cold resistance in the hardened condition and which exhibit a high degree of winter hardiness in the field also proved capable of building up higher dry-matter and sucrose content under controlled hardening than did those varieties of lesser cold resistance and winter hardiness. Whether the increase in sugar content during hardening confers cold resistance or is simply a result of conditions leading to the development of cold resistance is a debatable question. Certainly other physiologic changes likewise coincide with hardening. Too, it should not be assumed that under all growth conditions sugar and dry-matter content will be a reliable index of cold resistance. It has been demonstrated [3] that variation in nutrition may destroy this relationship.

[3] Unpublished data.

Temperature and light intensity are unquestionably major factors influencing the development of cold resistance in winter wheat. Hardening is likewise dependent upon such supplemental factors as the preceding stage and nature of growth. Since the response to temperature is a varietal characteristic, there is some justification for believing that the response to light intensity and supplemental factors may likewise be varietal in nature. Further, the studies show that the temperature response itself need not be the same for all parts of the plant. For instance, the leaves of one variety may show more ability to accumulate reserves than those of another, while the response of the crowns may be just reversed. While the cold resistance of the crown governs survival of the plant, it is obvious that leaf survival is of importance especially during the period when metabolic processes are gradually building the high levels of reserves attained by midwinter.

The foregoing observations help to explain the reason for the "reversals" often encountered in winter-hardiness nurseries. When two varieties of winter wheat are compared relative to their winter hardiness, as determined over a number of years, one variety may be found decidedly superior to the other. However, in certain years this position is reversed, the variety of high average winter hardiness being abnormally low. In view of the fact that climatic factors vary widely from year to year and that varietal winter hardiness must be dependent on such factors as speed of hardening, level of maximum attainable cold resistance, resistance to dehardening, response to variable light conditions, and others, it would be surprising if such reversals did not occur.

Controlled hardening studies, not being dependent on variable climatic conditions, should provide a means of predicting the average field behavior of a vareity. The present study indicates that this cannot be done merely on the basis of the relative content of carbohydrate reserves at any one time during hardening. For example, the relatively great field winter hardiness of Minhardi wheat may be the result not only of its ability to acquire a high level of cold resistance but likewise the result of rapidity of hardening and ability to retain cold resistance under dehardening influences. On the other hand, the low degree of winter hardiness which such varieties as Blackhull and Fulcaster exhibit in the field would be expected by reason of their lack of ability to develop a high level of reserves.

The data presented would seem to justify the use of sucrose or dry-matter content, or both, as a measure of the changes in cold resistance taking place under controlled uniform hardening or dehardening conditions. The use of controlled hardening in conjunction with either adequate freezing or chemical analysis should provide a valuable adjunct to nursery and controlled freezing technics in the determination of varietal winter hardiness.

SUMMARY

Controlled low-temperature hardening technic in conjunction with high-temperature dehardening was applied to the study of winter hardiness in wheat. Degree of cold resistance at any stage of hardening was measured either by controlled freezing or by analysis for dry-matter and sugar content. The ferricyanide method for sugar deter-

mination was successfully applied in a form permitting great rapidity of analysis.

The progressive increase of dry-matter and sucrose content over an 8-week controlled low-temperature hardening period was demonstrated. A parallelism was established between these increases and increases in cold resistance. Controlled hardening was shown to be comparable to natural field hardening, the morphological and physiological changes in the wheat plant being very similar for both. Light intensity was found to be a very important factor in hardening. Diminution of light intensity resulted in decreased efficiency of hardening as regards both cold resistance and storage of reserves. Under constant low-temperature conditions photoperiod was apparently not an important factor as plants hardened adquately under either increasing or decreasing day-lengths. Growth stage preceding hardening influenced the efficiency of hardening.

High-temperature dehardening, i. e., the loss of cold resistance during the period of transition from dormancy to active growth, proved essentially a reversal of the hardening process. Dry-matter and sugar content showed a progressive decrease with time of dehardening.

The cold resistance of neither the roots nor the leaf blades is a reliable index of the cold resistance of the plant. Survival seems to be entirely dependent on the ability of the crown to resist frost injury. However, the importance of leaf survival as contributing to the physiologic changes associated with the acquisition of cold resistance is evident.

Controlled low-temperature hardening and high-temperature dehardening studies were made on six varieties of winter wheat with the occasional inclusion of a variety of winter barley and one of spring wheat. Response to these conditions proved varietal in nature. There was good agreement between relative field-exhibited winter hardiness and the development of cold resistance and accumulation of reserves under controlled hardening. This was particularly true for the plant crowns. Leaf composition showed fair agreement with known cold resistance, this agreement being enhanced by a 2- or 3-day dehardening period. The studies suggest that observations of the trend of carbohydrate metabolism under the influence of controlled hardening and dehardening would prove of distinct value in determining the varietal winter hardiness of wheat.

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STRAINS OF CUCUMBER MOSAIC VIRUS PATHOGENIC ON BEAN AND PEA [1]

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INTRODUCTION

During a study of spotted wilt of garden pea (*Pisum sativum* L.) in the spring of 1935 (*36*),[3] an unusual development of symptoms atypical of that disease occurred in an out-of-door planting of peas at Madison, Wis. Mild mottling appeared on the younger leaves as well as a faint necrosis on stems, leaves, and pods. When juice extracted from certain of these plants was transferrred to tobacco (*Nicotiana tabacum* L. var. Havana 38), local and systemic symptoms of spotted wilt developed on a small percentage of the plants. On the remainder of the plants either local necrotic lesions or chlorotic areas occurred on the inoculated leaves, but the systemic mottle which followed was definitely not characteristic of the disease in question. Moreover, a contaminating virus was demonstrated by means of differential hosts. The mottle virus was isolated by inoculation to cucumber (*Cucumis sativus* L. var. White Spine) and the spotted wilt virus by inoculation to nasturtium (*Tropaeolum majus* L.)

Adjacent to the planting of peas was a number of rows of bean (*Phaseolus vulgaris* L.) which included the varieties Wisconsin Refugee and Idaho Refugee, known to be resistant to common bean mosaic (bean virus 1). As these plants came into blossom, about 5 percent of them showed symptoms similar to yellow bean mosaic (bean virus 2) (*21*). As the season progressed, however, the symptoms differed in many respects from those of the latter disease. In addition to marked stunting and various degrees of mottle, necrosis occurred on leaves, stems, and petioles. Some resemblance to bacterial blight (*Bacterium phaseoli* EFS) was occasionally suggested, but attempts to isolate this bacterial organism yielded negative results. Inoculation with expressed juice to pea, bean, cucumber, and tobacco resulted in mosaic on the last two species, while on pea the symptoms were mild necrosis and faint mottle and on bean a diffuse mottle and severe stunting.

These preliminary cross inoculations suggested that the diseases on pea and bean were possibly due to the same virus and that the latter was capable of bringing about symptoms on cucumber and tobacco not unlike those associated with cucumber virus 1. In the autumn of the same year (1935), M. W. Stubbs brought to the writers from a Madison greenhouse a young pea plant which showed mild stem necrosis. Inoculation to cucumber, tobacco, and pea resulted in infection on all three, while transfer to bean was unsuccessful. Since none

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[3] Italic numbers in parentheses refer to Literature Cited, p. 58.

of the mosaic or streak viruses heretofore isolated from pea or kidney bean, except the tobacco-ring-spot virus, had been shown to infect systemically both tobacco and cucumber, it was deemed essential to give the matter further attention. The present paper is a report on studies of the diseases and associated viruses which have been carried on at the Wisconsin Agricultural Experiment Station since 1935. A preliminary report (*37*) has already been published.

METHODS AND MATERIALS

The greenhouses in which studies were conducted were kept free of insects by frequent fumigation. In most cases the experimental plants were grown at temperatures most favorable for their normal development, and after inoculation they were held at 23° to 26° C. Insect transmission studies were conducted in separate compartments. Field symptoms on peas, beans, cucumbers, and squash (*Cucurbita pepo* L. var. Giant Summer Crookneck) were studied on experimental plots near Racine, Wis., during the summer of 1939.

Preliminary investigations were carried out with a number of isolates [4] from pea and bean. Since most of these isolates were similar, one was selected for further study as representative of those infectious to both pea and bean. This virus was obtained from a necrotic pea pod of the Wisconsin Perfection variety. The diseased plant was grown in an outdoor plot at Madison, Wis., and showed a necrotic flecking of the leaves and streaking of the stem. This isolate will be referred to as strain 14. The other strain used extensively in this investigation was that secured from a necrotic pea plant, mentioned above, collected in a Madison greenhouse by M. W. Stubbs. This is referred to as strain 17, and at the outset it was distinguished from strain 14 by the fact that it was not infectious to bean. Certain substrains of 14 were isolated for study; the procedure used is described later in the text.

For comparative purposes a stock of cucumber virus 1 was obtained from James Johnson, Wisconsin Experiment Station. Celery virus 1 was supplied by F. L. Wellman, United States Department of Agriculture; it was isolated originally by him from *Commelina nudiflora* L. in Florida. All stocks of the viruses were maintained in young hybrid tobacco plants (*Nicotiana tabacum* × *N. glutinosa* L.), those for each virus being segregated on shelves widely separated from other plants.

All greenhouse plants were inoculated in early stages of growth. Peas were more readily infected when the first leaves were inoculated before they had unfolded, while beans were usually inoculated by rubbing the unifoliate leaves about the time when the first trifoliate leaf was unfolding. Carborundum powder was used as an abrasive in transfer of the viruses, and the residue was washed from the rubbed leaves with a fine spray. This method was used in all studies of symptoms, host range, and properties.

EXPERIMENTAL RESULTS

REACTION OF BEAN

In greenhouse studies strain 14, strain 17, cucumber virus 1, and celery virus 1 were used to inoculate the following varieties or strains

[4] The term "isolate" is used in this paper to designate a virus from a given source without implying that it is necessarily a distinct strain.

of bean: Asgrow Stringless, Black Valentine, Stringless Black Valentine, Asgrow Valentine, Tendergreen, Early Stringless Green Pod, Giant Stringless Green Pod, Full Measure, Bountiful, French Horticulture, Ruby Dwarf Horticulture, Tennessee Green Pod, Wisconsin Refugee, Idaho Refugee, Corbett Refugee, Kentucky Wonder Pole (white-seeded), Navy White, Kentucky Wonder Pole (regular), Sure Crop Black Wax, Hodson Wax, Brittle Wax, Improved Kidney Wax, Pencil Pod Black Wax, Wardwell Kidney Wax, and Keeney Golden Wax. At least 2 series, each containing 5 to 25 plants, were inoculated from each variety. All varieties inoculated with strain 14 developed symptoms. This virus was successfully recovered by transfer to young tobacco plants of juice extracted from each of 16 varieties selected at random. Many attempts to recover the other 3 viruses were unsuccessful.

It is evident that strain 14 is distinctive in that it is the only one of the four viruses used which affects bean. Wellman (*33*) attempted to infect bean with celery virus 1, but likewise secured negative results. Harter (*7*) described a virus (believed to be related to cucumber virus 1) that was pathogenic on several varieties of lima bean (*Phaseolus lunatus* L.), but not on kidney bean or pea. The preliminary report by the writers (*37*) is apparently the first record of the successful inoculation of a virus in the cucumber mosaic group to kidney bean and pea. More recently Zaumeyer (*39*) has reported two strains of cucumber virus affecting pea, sieva lima bean, and broadbean (*Vicia faba* L.), but not kidney bean.

Although no variety was found immune to strain 14, some differences in expression of symptoms and in the amount of stunting, mottling, and necrosis occurred. In general, these symptoms were not sufficiently distinct to warrant placing the varieties in different classes as suggested by Pierce (*21*) for bean virus 1. The range of symptoms may be said to include about all those described for both bean virus 1 and bean virus 2. On the other hand, it is difficult to ascribe to strain 14 any one symptom or group of symptoms which might be sufficiently characteristic to set it apart from the other two viruses just mentioned. Four varieties representing the range of symptoms were selected for detailed study.

The unifoliate leaves of the Wisconsin Refugee develop a distinct drooping within 4 or 5 days after inoculation with strain 14. If these primary leaves are not fully developed at the time of inoculation, vein clearing and yellowing are also produced in them. The first trifoliate leaf next shows spinasty and vein clearing, with each leaflet twisted and curved slightly inward from the apex. As the leaf continues to develop, a diffuse yellow mottle appears, while the second trifoliate leaf develops a more distinct mottle of the same type (fig. 1, *C*).

The drooping of the unifoliate leaves serves to distinguish strain 14 from bean virus 2, which Pierce (*21*) described as causing a downward drooping of the first trifoliate. Since bean virus 1 does not affect the position of the leaves, epinastic response may be used to distinguish each of the three viruses in the early stages of development on plants inoculated in the simple leaf stage in the greenhouse.

Soon after the appearance of the first symptoms induced by strain 14, retardation in growth is evident (fig. 2). This difference becomes even greater with age and is frequently the chief means of distinguishing the diseased from the healthy plants. Retardation in develop-

ment of leaves may be seen in figure 1, *C*, *E*, which shows a healthy and a diseased leaf from Wisconsin Refugee plants of the same age. The petioles and the internodes are also shortened, and stimulation of axillary buds gives the plant a bushy appearance, expecially under field conditions.

In the same field in which strain 14 was originally isolated certain Wisconsin and Idaho Refugee plants developed a light yellow mottle

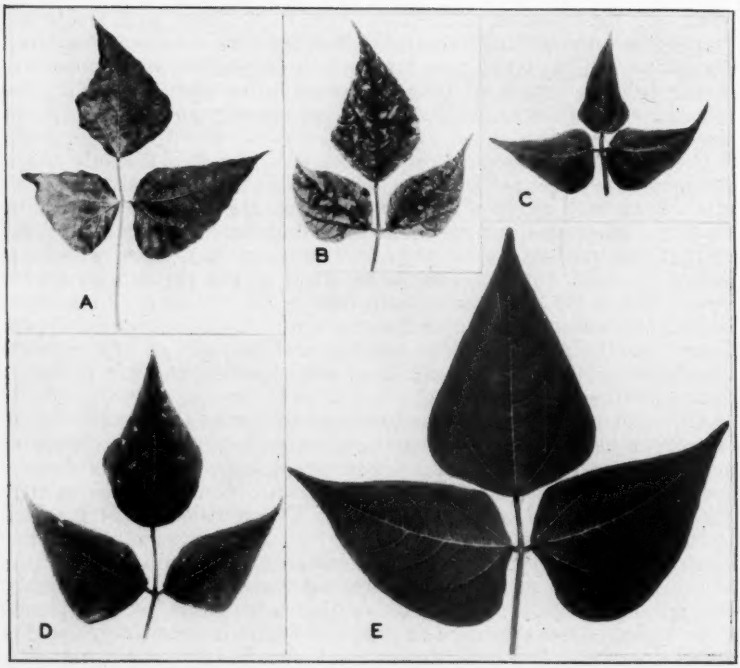

FIGURE 1.—Symptoms produced in 3 weeks on the second trifoliates of plants of four varieties of bean inoculated with strain 14: *A*, Mottling and wavy leaf margins in Hodson Wax; *B*, pronounced mottling, vein banding, and dark green islands in Sure Crop Black Wax; *C*, diffuse mottle, slight curling of apices, and extreme reduction in size in Wisconsin Refugee; *D*, chlorosis near margins and deeper than normal green in the region of the midvein in Full Measure; *E*, uninoculated leaf of Wisconsin Refugee. Note marked reduction in size of all inoculated leaves.

similar to that described for bean virus 2 (*21*). Isolations from these plants consistently produced symptoms on both tobacco and beans. Since under greenhouse conditions the inoculated bean plants later outgrew the leaf mottle symptoms, it was concluded that bean virus 2 was probably not present in these plants and that the field symptoms were due to strain 14. Other plants in this field showed necrosis of leaves, petioles, and stems, a symptom which was especially prominent at the pulvinus and growing tip. It is now believed that one or more yellow strains of 14 were present in this field, since these necrotic symptoms are similar to those later produced with such strains of the virus.

Further field trials were conducted during the summer of 1939 near Racine, Wis. Three 50-foot rows of Wisconsin Refugee were planted, and when the third trifoliate was unfolding the first 20 plants of each of two rows were inoculated with strain 14. One month later the infected plants showed marked stunting but had not developed a definite mottle. The only other signs of disease were the irregular glossy surface and vein clearing of younger leaves. An occasional uninoculated plant in each of the three rows also showed symptoms similar to those which had been inoculated. Transfer of inoculum from these plants to tobacco demonstrated that they were also infected with strain 14. By the end of the season 90 percent of the uninoculated plants had become infected. In no case was mottling or necrosis very evident; marked

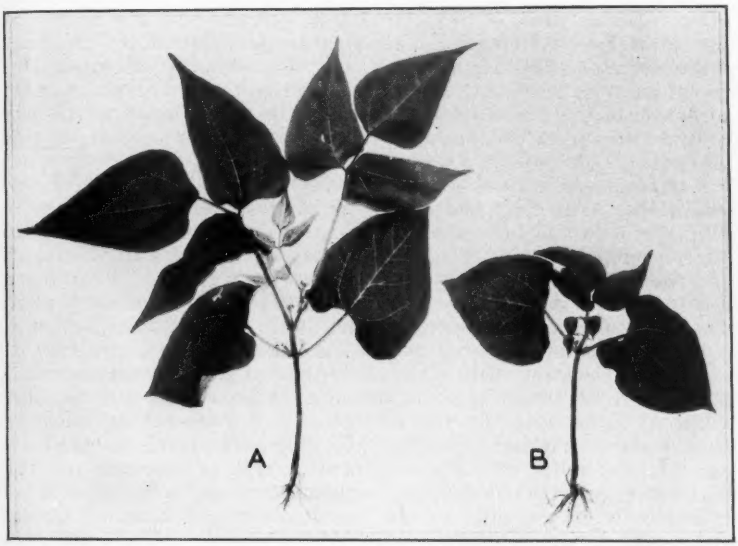

FIGURE 2.—*A*, Uninoculated Wisconsin Refugee bean plant; *B*, plant of same age as that in *A*, inoculated 3 weeks previously with strain 14. Note extreme retardation in growth.

stunting and the glossy, wavy appearance of the leaves were the only distinguishing symptoms. As compared with the few remaining healthy plants, the production of pods on these diseased plants was greatly reduced, and small misshapen pods were more common. The natural spread of the virus was probably brought about by aphids. The potato aphid, *Macrosiphum* (*Illinoia*) *solanifolii* (Ashm.), was abundant and an occasional peach aphid, *Myzus persicae* (Sulz.), was observed. Although the capacity of the potato aphid to transmit strain 14 has not been investigated, it is an established vector of cucumber virus 1, while *M. persicae* is shown later in this paper to be a vector of strain 14.

Considerable variation in symptoms has been shown to occur on Wisconsin Refugee in the greenhouse and in the field. Even greater variations occur between different varieties. On Sure Crop Black

Wax, symptoms are more pronounced than on any of the other varieties tested and they closely resemble those produced by bean virus 2. Initial symptoms are much like those produced on Wisconsin Refugee, with drooping of the unifoliates and vein clearing of the first trifoliates, the latter condition usually changing gradually to vein banding. Light yellow areas soon develop and gradually spread to all parts except along the larger veins, which remain dark green (fig. 1, *B*). Occasionally, the vein banding symptom does not persist and the mottle is more like that of bean virus 2, except that the yellowing is less extensive. Scattered small dark green islands commonly occur. The plants are stunted to about the same degree as those of Wisconsin Refugee.

On Full Measure the symptoms on the first and second trifoliate often resemble those of bean virus 1, as described by Pierce (*21*) on Stringless Green Refugee. Vein clearing is followed by chlorosis, especially along the edges (fig. 1, *D*). On some leaves small dark green areas appear scattered promiscuously over the lighter green surface or concentrated along the larger veins. These symptoms may continue to appear on developing leaves, but usually become entirely masked. Stunting is less severe than in Wisconsin Refugee.

Symptoms on Hodson Wax are usually very mild under greenhouse conditions. The first and second trifoliates usually show a mild, diffuse mottle and occasionally darker green areas (fig. 1, *A*). On later-developing leaves it is often impossible to detect any mottling, and the leaf may show only an irregular, glossy surface. Stunting is less marked than with the varieties described above, and it is often necessary to transfer the virus to other hosts to ascertain infection.

In general, stunting is the most constant symptom of strain 14 on the bean varieties studied. The slower rate of growth causes a reduction in size of leaves and a shortening of internodes and petioles. Stunting varies with the variety, but it is a more constant feature than in the case of bean virus 1. Mottling of the leaves is extremely variable, depending on the environment as well as the variety. The slightly irregular, glossy surface is a rather constant feature but is too indistinct to be detected by the casual observer. Masking of leaf symptoms in the greenhouse and field is common. More extensive field observations are needed to determine the most constant field symptoms.

The reaction of the bean to various "yellow" and "dark green" substrains of 14 is discussed later in this paper.

REACTION OF PEA

Three varieties of pea were inoculated with the same four viruses used in the bean studies. The results (table 1) show that symptoms appeared in all cases except in the plants of the Wisconsin Perfection variety inoculated with cucumber virus 1 and celery virus 1. Furthermore, in the case of the Alderman variety local lesions only were produced after inoculation with celery virus 1. In no case was cucumber virus 1 or celery virus 1 recovered from inoculated pea plants even though symptoms had occurred. Strains 14 and 17, on the other hand, were recovered quite readily.

The symptoms which develop on young plants inoculated and grown in the greenhouse will be described first.

TABLE 1.—*Comparative symptoms on three varieties of pea inoculated with strain 14, strain 17, cucumber virus 1, and celery virus 1*

Variety	Strain 14		Strain 17		Cucumber virus 1		Celery virus 1	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Alderman	137	82	55	47	20	24	10	[1] 20
Alaska	74	62	28	50	12	[2] 8	14	21
Wisconsin Perfection	88	49	27	52	30	0	17	0

[1] Only local lesions were produced.
[2] The only symptom was stem necrosis at the base of the inoculated leaf.

STRAIN 14

When young pea plants of the Alderman variety are inoculated before the first leaves have unfolded, the initial symptoms appear in 4 or 5 days as a wilting or necrosis of some of the rubbed leaves and stipules. In 6 to 10 days there appear on the youngest leaves diffuse, yellow-green areas which may develop into a more definite mottle (fig. 3, *B*), or may disappear entirely. The developing leaves on infected plants tend to remain folded and slightly twisted. A pronounced stunting soon follows, with reduction in size of leaves and shortening of internodes as the most conspicuous symptoms (figs. 4 and 5). The lower leaves continue to wilt and die, and under greenhouse conditions 35 to 50 percent of the plants of this variety develop faint, but definite, watery, purple-brown, necrotic streaks on the stem. Necrosis may advance up one side of the stem or affect the entire plant, including leaves and growing tip. Leaf necrosis is usually most evident along the veins. If the growing tip is killed, new shoots may develop in the axes of the lower leaves, but they remain stunted and often show mottling. Infected greenhouse plants may persist for some time but eventually die prematurely.

On the Alaska variety symptoms are similar to those on Alderman except that stem necrosis is more extensive. Symptoms on Wisconsin Perfection frequently appear as diffuse necrotic areas on the leaflets and occasionally as a streaking on the petioles and stem. Stem necrosis is more pronounced and mottling more distinct than on the other two varieties. The killing of the growing tip is also common. Symptoms produced by substrains of the virus on this variety are described later in this paper.

In the field two 50-foot rows each of Alderman and Wisconsin Perfection were planted, and 4 weeks later 20 plants in one row of each variety were inoculated mechanically with strain 14. Mottling of some plants and stunting of others were evident in 2 weeks, and many later developed a mild necrosis of the stem and growing tip. At 5 weeks all inoculated and a few uninoculated plants had developed the disease. By the end of the season 95 percent of all the plants were infected, and isolations from representative plants gave only strain 14. Since the potato aphid could be found on almost every plant and the peach aphid on an occasional one, it is probably that one or both of these species spread the virus from the artificially infected plants.

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Initial symptoms on naturally infected plants were similar to those described above except that death did not often occur as promptly. However, the main shoots usually died and secondary branches developed near the base of the plant. The rosettelike appearance of

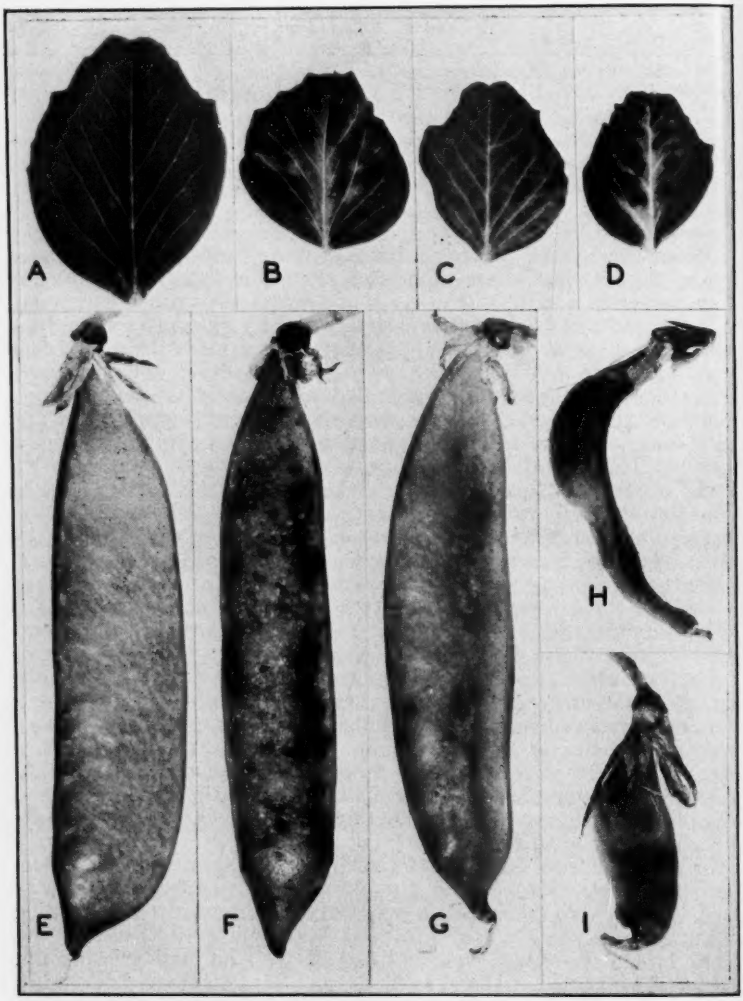

FIGURE 3.—Symptoms on leaflets and pods of peas inoculated with strain 14: *A*, Leaflet from uninoculated Wisconsin Perfection plant; *B*, leaflet from Alderman inoculated with strain 14 (note diffuse mottle); *C*, *D*, leaflets from Wisconsin Perfection inoculated with yellow strain of 14 (mottle is more distinct than in *B*); *E*, pod from uninoculated Alderman; *F*, *G*, necrosis of pods from field-inoculated Alderman plants; *H*, *I*, twisting and stunting of younger pods from the same variety.

these shoots (fig. 5) was due to extreme shortening of the internodes and distortion of the leaves. Mottling of leaves and necrosis of stems were less common on secondary branches, but necrotic flecking of leaves and stems usually developed. Blossoms which were well

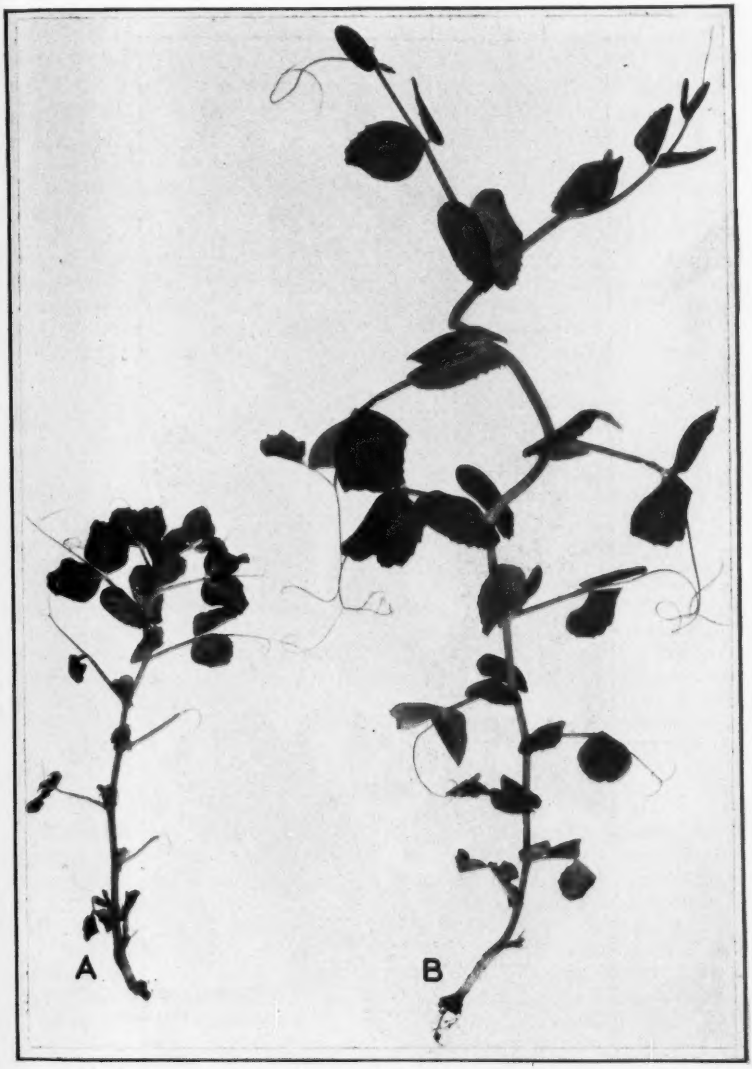

FIGURE 4.—Wisconsin Perfection pea plant (*A*) 3 weeks after inoculation with strain 14. Note extreme reduction in size as compared with uninoculated plant (*B*), as well as necrosis of lowest leaves and mottle of young leaflets.

developed at the time of initial symptoms usually dropped, while small pods became distorted and necrotic (fig. 3, *H*, *I*). Some older pods remained quite normal, while others showed severe necrosis (fig. 3, *F*, *G*), and peas from the latter were occasionally mottled or necrotic. On the secondary branches only a few pods set, and these

FIGURE 5.—Portion of an Alderman plant naturally infected in the field with strain 14. The internodes of side branches remain short, and faint necrosis occurs along the veins of some leaflets.

either developed normally or showed symptoms as described above. The virus was readily recovered from stem, leaves, and pods.

Strain 17

The symptoms of this virus closely resemble those described above for strain 14 on the same three pea varieties. Stem necrosis, however, is more pronounced and vein clearing of the young leaves is more common than mottling. Stunting is equally severe, and plants showing necrosis usually die prematurely.

Cucumber Virus 1

In no case has this virus been recovered from inoculated pea plants, although a few such plants have shown definite symptoms. In a 10-plant series of Alderman, 1 plant developed a necrosis of rubbed leaflets and petioles; in a few days necrosis had advanced 1 cm. along the stem. Besides this necrosis, stunting was the only additional symptom noted on this plant. In another plant in the same series distinct stem and top necrosis occurred, followed by early death of the plant. In still another series of this variety faint necrotic streaking developed on stems of 3 plants. Out of 12 inoculated plants of the Alaska variety, one showed stem necrosis at the base of an inoculated leaf. In 3 separate series inoculation to Wisconsin Perfection gave negative results. From these experiments it is doubtful if pea should be considered a host of the strain of cucumber virus 1 used, but it is indicated that the virus may occasionally enter and progress within the tissue.

Celery Virus 1

Similar preliminary results indicated that this virus may also go to peas. In one series of 10 Alderman plants necrosis of inoculated leaflets and stipules developed on 2 without the appearance of systemic symptoms. In another 4-plant series of Alaska speckle necrosis of leaflets and stipules occurred on 3 of the plants. No attempt was made to recover the virus. In 3 separate series inoculation to Wisconsin Perfection failed to produce symptoms. Further studies are needed to determine whether peas may be considered a host of this virus.

Substrains of Virus 14

Tobacco leaves infected with strain 14 occasionally showed bright yellow areas on the older leaves (fig. 6, *A*) similar to those described by McKinney (*20*) for common tobacco mosaic and by Price (*24*) for cucumber mosaic; from these areas "yellow" substrains were isolated (fig. 6, *B*, *C*). In addition, two other substrains, "dark green" and "normal green," were isolated from tobacco leaves infected with strain 14. The "dark green" isolate was obtained from a small raised area of tissue darker than normal green. The other was from a nearly normal green area on the same leaf.

On tobacco the yellow substrains produced amounts of chlorosis varying with the isolate (fig. 6, *B*, *C*). Mottle was accompanied by much leaf distortion and stunting. One of these isolates (fig. 6, *B*) was used to inoculate cucumber, Wisconsin Refugee bean, Wisconsin Perfection pea, and Black cowpea (*Vigna sinensis* Endl.) in the green-

house. On cucumber the small circular chlorotic primary lesions rapidly spread to produce a yellowing and quick death of the cotyledons. Chlorotic sunken streaks next appeared on the hypocotyl.

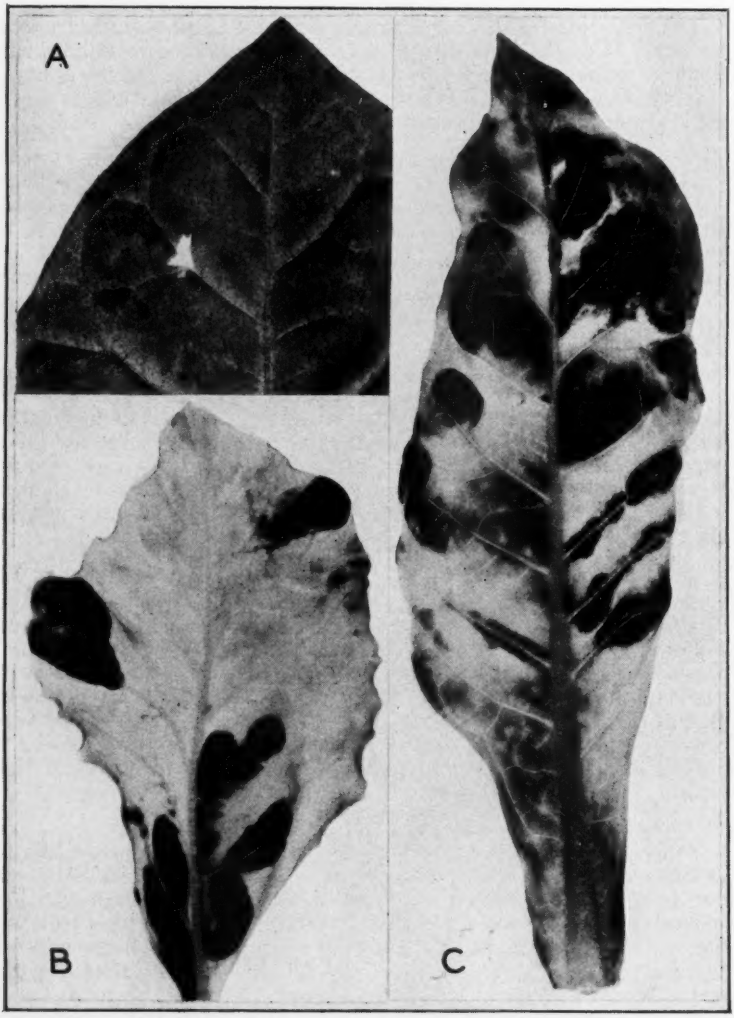

FIGURE 6.—*A*, Bright yellow spot in a tobacco leaf infected with strain 14. From the tissue of such spots "yellow" strains were isolated. *B*, *C*, Leaves from tobacco plants infected each with a different "yellow" isolate of strain 14.

followed by a yellowing of the true leaves. Infected plants usually died soon after systemic symptoms appeared. Initial symptoms on Wisconsin Refugee bean and Black cowpea were epinasty of the rubbed unifoliate leaves followed by chlorotic areas on these leaves.

Systemic symptoms frequently developed as a severe necrosis of stems, petioles, and leaves. Necrosis of the pulvinus was especially prominent. Most of these plants died prematurely. On those which survived or did not show necrosis a bright yellow mottle of the leaves and severe stunting of the plants developed. The mottle symptoms on cowpea were similar to those described by Price (*24*)) for his Y_1 and Y_2 strains. Wisconsin Perfection peas infected with the yellow substrains show more distinct yellow mottle (fig. 3, *C* and *D*), more leaf distortion, severe necrosis, stunting, and a higher mortality than do those infected with the original strain.

The dark green substrain was also compared directly with the other substrains. On tobacco it showed a mild but distinct mottle of dark green raised areas dispersed on a normal green background, and distortion and stunting were less severe. In general these symptoms were also typical of the dark green isolate on cucumber, Black cowpea, Wisconsin Refugee bean, and Wisconsin Perfection pea. The normal green substrain produced symptoms on the above hosts identical with those of the original strain. In all cases the yellow substrain produced the most severe symptoms, while the dark green strain was milder than any of the substrains or the original isolate.

From these experiments it is evident that, as has been reported for the cucumber mosaic virus (*24*), it is possible to isolate a number of substrains which remain stable, give distinct symptoms, and differ from one another in their severity on the respective hosts.

RELATION OF TEMPERATURE TO SYMPTOM EXPRESSION

Idaho Refugee, Kentucky Wonder Pole (regular), and Full Measure beans, and Alderman and Wisconsin Perfection peas were included in a study of the relation of temperature to expression of symptoms. After inoculation with strain 14 by the usual method, 10-plant groups of each variety were placed in temperature-controlled greenhouses at 16°, 20°, 24°, and 28° C. An equal number of check plants was placed at each temperature.

Epinasty of the unifoliate leaves was the initial symptom on bean at all temperatures, but time of appearance varied from 2 to 3 days after inoculation on plants at 28° C. to a week or more at 16°. Diffuse yellow areas next developed on the unifoliate leaves of plants at 28°, and in 5 days vein clearing and distinct mottle appeared; at the end of 2 weeks severe necrosis of stems, leaves, and growing tip had developed on 20 percent of the plants. The progress of the disease in plants at 24° was somewhat slower, and at 2 weeks vein clearing, mottle, and stunting, but no necrosis, were evident. The mottle developed still more slowly at 20°, but at 2 weeks the disease had advanced nearly as far as at 24°. At the end of the experiment the only symptom on plants at 16° was epinasty of the inoculated primary leaves.

Infected Wisconsin Perfection pea plants first showed mottling of the leaves at 24° C. in about 11 days, and stunting of the inoculated plants at all temperatures was then evident. In one experiment, 18 days after inoculation, 9 of the 10 plants at 28° were either dead or severely wilted and a few showed a faint necrosis of the stem; at 24° 4 of the 10 plants had developed faint necrosis of the stem and had died prematurely; at 20° typical mottling developed on 6, and on 1

necrosis of the growing tip occurred; at 16° none of the plants showed symptoms other than stunting.

Although the disease is most severe in its effect on peas and beans at 28°, the most typical symptoms develop at 24°. At 20° they occur more slowly and with less severity, although they are nevertheless typical in appearance. Symptoms other than epinasty are completely masked at 16° C., but the virus is readily recovered from inoculated plants held at this temperature.

PROPERTIES OF THE VIRUSES

The properties of strains 14 and 17 were studied to determine how closely they conform to those reported by Johnson (*15*), Hoggan (*10*), and Price (*24*) for cucumber mosaic viruses and by Wellman (*33*) for celery virus 1. In the matter of longevity in vitro cucumber virus 1 used in other studies reported herein was compared with strains 14 and 17.

Expressed juice from tobacco plants recently infected with the viruses was treated and then used to inoculate tobacco as the test plant. Ten plants were used as a test unit and each experiment was repeated at least once (table 2).

TABLE 2.—*Comparison of certain properties of cucumber virus 1, strain 14, and strain 17 as determined by inoculation of tobacco* [1]

Thermal inactivation point					Longevity in vitro							Tolerance to dilution				
Temperature (° C.)	Strain 14		Strain 17		Time aged (days)	Strain 14		Strain 17		Cucumber virus 1		Dilution	Strain 14		Strain 17	
	Trial		Trial			Trial		Trial		Trial			Trial		Trial	
	1	2	1	2		1	2	1	2	1	2		1	2	1	2
Untreated	10	10	10	10	None	10	9	10	10	10	10	None	10	5	9	9
45°	7	9	10	10	1	10	----	9	----	----	----	1–10	10	6	7	4
50°	6	9	10	10	2	10	----	9	----	----	----	1–100	9	2	6	2
55°	5	5	7	7	3	9	----	4	----	----	----	1–1, 000	2	1	4	1
60°	10	3	3	1	4	9	5	2	8	10	10	1–10, 000	1	0	0	0
65°	5	0	1	0	5	10	3	4	6	4	8	----------	----	----	----	----
70°	0	0	1	0	6	9	1	0	6	1	6	----------	----	----	----	----
75°	0	----	0	----	7	10	1	0	1	0	1	----------	----	----	----	----
----------	----	----	----	----	8	6	0	0	0	0	0	----------	----	----	----	----
----------	----	----	----	----	9	0	----	0	----	0	----	----------	----	----	----	----
----------	----	----	----	----	10	1	----	0	----	0	----	----------	----	----	----	----

[1] Figures represent the number of tobacco plants infected out of 10 inoculated with the virus indicated.

THERMAL INACTIVATION POINT

The method used for heating the virus samples was the same as that described by Price (*23*) and later used by him in comparing yellow strains of cucumber mosaic (*24*). The thermal inactivation points of strains 14 and 17 were found to be between 65° and 70° C., when exposed at this temperature for 10 minutes. These figures are in close agreement with those reported by Hoggan (*10*) for cucumber mosaic viruses and are within the range given by Johnson (*15*) for classification of the cucumber virus group. Price (*24*), using the same method, obtained a variation from 64° to 72° for eight different strains, but concluded that they were essentially similar in their resistance to heating. Wellman (*33*) found that celery virus 1 occasionally

remained infective after treatment at 75°, but that it was inactivated in all samples treated at 80°.

Longevity in Vitro

The expressed juice from aging studies was stored in stoppered test tubes at 20° to 22° C. The longevity in vitro was 7 to 8 days for strains 14 and 17. In one trial, however, one plant became infected by inoculum aged for 10 days, although none were diseased after inoculation with the same extract at 9 days. These results are in general accord with those for cucumber virus 1, which was found to lose its infectivity at 6 to 7 days, though Johnson (*15*) and Hoggan (*10*) report 3 and 5 days, respectively, for this virus.

Tolerance to Dilution

Dilutions were made with sterile distilled water and the plants were inoculated immediately. There was no significant difference between strains 14 and 17. The maximum dilution at which infection occurred was usually 1–1,000, although in one case it was 1–10,000. These results are in general agreement with those reported by other workers for the cucumber mosaic virus group (*10*, *33*), which ranges from 1–1,000 to 1–100,000 in maximum dilution from which infection may be secured.

Insect Transmission

The green peach aphid, *Myzus persicae* Sulz., was studied as a possible vector of strains 14 and 17. Nonviruliferous aphids were raised on healthy cabbage plants and later transferred to a variety of infected hosts, on which they were allowed to feed from 48 to 72 hours. At the end of this period 10 to 15 aphids were transferred to each healthy plant to be infected. After another feeding period of 48 to 60 hours the aphids were killed by fumigation. In the transfer of a small number of insects a brush of red sable's hair was used, but for larger numbers the leaf method of Hoggan (*8*) was found useful.

Strain 14 was transferred by means of aphids from tobacco to tobacco, yellow sweetclover (*Melilotus officinalis* (L.) Lam.) to pepper (*Capsicum annuum* L. var. California Wonder), kidney bean to pepper, spinach (*Spinacia oleracea* L. var. Bloomsdale) to pepper, pepper to pepper, and pea to jimsonweed (*Datura stramonium* L.). Strain 17 was also transmitted by the same vector from pea to spinach and from spinach to spinach. Six plants served as a test unit in each case. The virus was recovered from all hosts except jimsonweed. The incubation period in the hosts varied from 14 to 21 days. No experiments were conducted to demonstrate the length of time required for the aphid to become infected or the length of time it remained infective.

Seed Transmission

Transmission of bean virus 1 through the seed of common bean was first demonstrated by Reddick and Stewart (*29*), but bean virus 2 was found nontransmissible by Pierce (*21*). Doolittle and Gilbert (*5*) have given evidence to prove that cucumber mosaic may be transmitted through the seed of the wild cucumber (*Micrampelis lobata* (Michx.) Greene), and Kendrick (*16*) demonstrated occasional transmission of the same virus through the seed of muskmelon varieties

Honey Dew and Persian (*Cucumis melo* L. vars. *inodorus* and *reticulatus* Naud.).

In a total of 580 pea and 440 bean seedlings grown from seed of plants infected with strain 14 none was found to be affected with the virus. The pea and bean seeds used were obtained from both naturally and artificially infected field plantings reported earlier in this paper. Further trials are needed to determine conclusively whether this virus is transmitted through pea and bean seed, but these preliminary trials suggest that it is not. No seed transmission studies with strain 17 have been made.

HOST RANGE

Rather than ascertain the infectivity of strains 14 and 17 on a series of plant species chosen at random, it was considered more to the point to make a study on known hosts of the cucumber mosaic group of viruses. This list includes those plants most commonly infected in nature with cucumber virus 1 and, secondly, those which have been more recently reported as hosts of this virus or strains closely related to it. As strains 14 and 17 were first observed on peas and beans, other legume hosts, such as lima bean, cowpea, lupine (*Lupinus angustifolius* L. and *L. hartwegii* Lindl.), and sweetclover were of special interest. A list of the plant species susceptible to one or more of the four viruses studied in this paper and the cardinal symptoms produced on each host are given in table 3. This list includes 19 species belonging to 16 genera and 6 families. In addition none of the four viruses was found to be infectious on White Dutch Clover (*Trifolium repens* L.) or soybean (*Soja max* (L.) Piper var. Manchu). From 5 to 50 plants or more of each species were inoculated, and with few exceptions the virus was recovered when signs of disease developed.

A study of table 3 will reveal that all plants susceptible to cucumber virus 1 are also hosts of strain 14, and, in addition, the latter virus brought about infection on Fordhook Mammoth Pod lima bean and yellow sweetclover. Broadbean, susceptible to celery virus 1, was the only plant tested not infected by strain 14. The celery virus also produced infection on all plants tested except Fordhook Mammoth Pod lima bean and yellow sweetclover. The host range of strain 17 paralleled that of cucumber virus 1 and in addition included yellow sweetclover.

It is not the purpose of this paper to describe in detail the symptoms on all the hosts infected with different viruses. However, it should be mentioned that in general the symptoms on the respective hosts were similar as to type, and any variations between viruses were mostly in degree or intensity. For instance, under field conditions, cucumber and squash infected with strain 14 not only produced plant symptoms identical with those of cucumber virus 1, but also gave rise to wartlike areas and distortion of the fruit. The similarity of the mottle symptoms of the four viruses may be seen in figure 10, while typical variations in these symptoms are shown on tobacco in figure 7. It is at once apparent that symptoms produced by strain 17 on this host are much milder than those of the other two viruses. Symptoms of strain 17 (fig. 7, *C*) most closely resemble those of "cucumber mild mosaic virus" described by Hoggan (*10*).

Strain 17 was somewhat unstable and occasionally symptoms similar to those of cucumber virus 1 appeared on tobacco. Transfer of the virus from such plants give rise to stable substrains. Strain 14 produced many types of symptoms on tobacco leaves, including oak-leaf patterns, savoy, and reduction of leaf lamina. The principal difference in this regard between strain 14 and cucumber virus 1 was a slightly greater amount of yellow in the lighter areas of the mottle pattern produced by the former.

On cowpea, primary lesions were produced by all four viruses (fig. 13, *A*) similar to those described by Price (*24*). The necrotic lesions produced by cucumber virus 1 were slightly larger and more numerous than those of the other three viruses. In addition, strain 14 produced other primary symptoms, such as circular chlorotic areas which later often became partly necrotic (fig. 13, *B*, *C*) and had a tendency to spread out along the main veins. Systemic symptoms, such as mottling, leaf distortion, bronzing (fig. 13, *D*), and severe stunting, developed on this host (fig. 14, *B*).

Dark necrotic lesions were produced on the inoculated primary and true leaves of watermelon by cucumber virus 1 and strains 14 and 17 not unlike those described by Wellman (*35*) for celery virus 1. The yellow halo about the necrotic lesions was usually conspicuous and on one plant inoculated with strain 14 a yellowing developed over the entire plant. An attempt to recover the virus was unsuccessful.

The infection of Henderson Bush lima by all four viruses is of interest in view of Harter's (*7*) report that a strain of cucumber mosaic was infectious to eight varieties of small-seeded limas but not to the large-seeded Fordhook types tested. Of the writers' four viruses only strain 14 infected Fordhook Mammoth Pod. The symptoms of the four viruses on Henderson Bush lima were similar and developed as epinasty, vein clearing, mottling, stunting, and occasionally bronzelike necrosis of leaves and necrosis of stems and growing tip. Strain 14 produced the most severe symptoms of the four viruses and death of the plants was common. The symptoms observed by the writers on this host are not unlike those described by Harter (*7*) except that the latter did not mention any necrotic condition. Since cucumber virus 1, as well as the other three strains used in this study, infects Henderson Bush lima bean, it is obvious that this is not a good differential host for strains of the cucumber virus group.

The serious disease that developed from inoculating lupine with strains 14 and 17 and cucumber virus 1 is similar to the lupine disease reported from Germany by Köhler (*17*, *18*), who considered it to be identical with Ainsworth's (*1*) "yellow-mottle mosaic" (cucumber virus 1 of Johnson).

Wellman (*33*) reported local lesions on leaves of broadbean inoculated with celery virus 1 but did not obtain systemic infection. In the course of the experiments reported herein typical local lesions were also obtained with this virus, and in a few cases systemic symptoms also developed.

Inoculations of strain 14 to perennial chrysanthemum gave symptoms which suggest a similarity to those of other viruses on this plant as reported by Valleau (*31*) and Burnett (*2*), although the reaction of the viruses described by these investigators was different on other hosts from that of the four studied here.

TABLE 3.—*"Type symptoms" as produced on various hosts by cucumber virus 1, together with variations from "type symptoms" produced by celery virus 1, strain 14, and strain 17*

Species and common name	Type symptoms[1] (cucumber virus 1)	Variations from type symptoms as described for cucumber virus 1		
		Celery virus 1	Strain 14	Strain 17
Nicotiana tabacum L. (tobacco—var. Havana 38).	Local: Occasional diffuse chlorotic areas in 7 to 9 days. Systemic: Vein clearing in 7 to 10 days; mottling, distortion, stunting (fig. 7, *B*).	Local: Small circular, bleached areas. Systemic: Similar (to type symptoms).[1]	Local: Usually chlorotic areas. Systemic: Similar, but with more yellow mottle (fig. 7, *A*).	Local: Same as strain 14. Systemic: Faint mottle, irregular surface; no distortion or stunting (fig. 7, *C*).
Nicotiana glutinosa L.	Local: Usually chlorotic areas. Systemic: Similar to symptoms on tobacco.	Local: Similar to symptoms on Havana tobacco. Systemic: Similar.	Local: Faint chlorotic areas. Systemic: Similar; also netlike mottle and more puckering.	Local: Same (as type symptoms).[1] Systemic: Netlike mottle remaining diffuse; no distortion or stunting.
Lycopersicum esculentum Mill. (tomato—var. John Baer).	Systemic: Vein clearing, mottle, distortion, stunting, filiform leaves.	Systemic: Mild mottle; leaf distortion uncommon.	Systemic: Diffuse chlorotic mottle (fig. 8, *B*).	Systemic: Indistinct, reticulate mottle; later symptoms often masked.
Datura stramonium L. (jimsonweed).	Local: Chlorotic areas (5 to 20 mm. diameter); later necrotic or bleached.	Local: More diffuse than type symptoms.	Local: Same. Systemic: Vein clearing, dark green mottle; distortion, stunting (fig. 9, *D*).	Systemic: Diffuse mottle; dark green, circular patterns.
Capsicum annuum L. (pepper—var. California Wonder).	Systemic: Mottling, distortion, stunting.	Systemic: Similar to strain 14.	Systemic: Similar to type; also dark green islands; yellowing, necrosis (fig. 9, *C*).	Systemic: No stunting or distortion.
Cucumis sativus L. (cucumber—var. White Spine).	Local: Occasional diffuse chlorotic areas on cotyledons. Systemic: Vein clearing, mottle, cupping, dark green areas, puckering, stunting (fig. 10, *C*).	Local: Chlorotic areas 3 to 4 mm. diameter; in 3 to 7 days necrotic. Systemic: Similar (fig. 10, *A*).	Local: Similar to celery virus 1, but later enlarge.. Systemic: Similar; also chlorotic streaking on stems (fig. 10, *D*).	Local: Similar to strain 14, but less necrosis. Systemic: Similar to but more severe than strain 14 (fig. 10, *B*).
Cucumis melo L.—vars. *reticulatus* Naud. and *inodorus* Naud. (muskmelon—vars. Rocky Ford and Honey Dew, respectively).	Systemic: Mottling, puckering, stunting (fig. 11, *A*).	Systemic: Same.	Systemic: Also chlorotic blotchlike areas; necrosis of older leaves (fig. 11, *B*).	Systemic: Yellow mottling, circular chlorotic areas; necrosis of leaf margins.
Cucurbita pepo L. (squash—vars. Giant Summer Crookneck and Cocozelle).	Local: Chlorotic areas on cotyledons (3 to 5 mm. diameter); few later became necrotic. Systemic: Vein clearing; first chlorotic blotches, later mottling, stunting, savoy, mottling, and distortion of fruit.	Local: Similar. Systemic: Similar (Wellman (*55*)).	Local: Similar (see fig. 12, *D*, *E*, *F*). Systemic: Less savoy and distortion (fig. 11, *C*).	Local: Same. Systemic: Milder, more diffuse, blotchy type mottle; less stunting and distortion (fig. 11, *D*).
Citrullus vulgaris Schrad. (watermelon—vars. Kleckley Sweet and Tom Watson).	Local: Chlorotic lesions on cotyledons later necrotic, spreading (fig. 12, *C*); similar lesions also on true leaves with yellow halo. Systemic: None.	Local: Black necrotic local lesion. Systemic: Vein clearing, mild mottle, stunting (Wellman (*55*)).	Local: Lesions smaller, less necrotic spreading (fig. 12, *B*).	Local: Same as for strain 14 (fig. 12, *A*).

Phaseolus lunatus L. (lima bean—var. Henderson Bush).	Local: Diffuse chlorotic areas on unifoliates, later brick-red necrosis spreading along veins. Systemic: Vein clearing, mild yellow mottle; necrosis of young leaves and growing tip; stunting.	Local: Same. Systemic: Same	Local: Similar. Systemic: Slightly more severe.	Local: Occasional small brick-red necrotic lesion. Systemic: Diffuse mottle; bronzing of leaves; necrosis of stem.
Phaseolus lunatus L. (lima bean—var. Fordhook Mammoth Pod).	No infection	No infection	Local: Chrolotic areas, later necrotic. Systemic: Necrosis in leaves and stems; premature death.	No infection.
Vigna sinensis Endl. (cowpea—var. Black).	Local: Dark-red necrotic lesions on unifoliate leaves (2 to 3 mm. diameter) in 2 to 3 days (fig. 13, *A*).	Local: Similar	Local: Fewer necrotic lesions; chlorotic areas, later partly necrotic; spreads mostly along veins (fig. 13, *B*, *C*). Systemic: Vein clearing, mottling, necrosis, distortion, stunting (figs. 13, *D*; 14, *B*).	Local: Few necrotic lesions.
Lupinus angustifolius L. and *L. hartwegii* Lindl.	Local: Occasional bronzelike necrotic areas. Systemic: Yellowing, faint mottle, necrotic flecking, dropping of leaves, stunting, stem necrosis, death (fig. 15, *C*).	Not tested	Local: Similar. Systemic: More yellowing, severe stunting (fig. 15, *B*).	Systemic: Similar to but milder than strain 14.
Melilotus officinalis (L.) Lam. (yellow sweetclover).	No infection	No infection	Systemic: Vein clearing, mild mottling, yellowing, necrotic flecking and dropping of leaves; stunting.	Systemic: Similar to strain 14, but less stunting and often no symptoms.
Vicia faba L. (broadbean)	do	Local: Dark brown lesions in 8 days. Systemic: Yellowing or necrosis of leaves and stems; stunting; death.	No infection	No infection.
Spinacia oleracea L. (spinach—var. Bloomsdale).	Systemic: Mild mottle, yellowing, severe puckering, malformation, and stunting.	Local: Same as for strain 14. Systemic: Same as type symptoms.	Local: Diffuse chlorotic areas. Systemic: Similar to type symptoms (fig. 9, *B*).	Systemic: Fine mottle, yellowing and irregular leaf surface.
Apium graveolens L.—var. *dulce* DC. (celery—var. Golden Self-blanching).	Systemic: Mild mottle, filiformity, raised blisterlike areas, stunting.	Systemic: More brilliant yellow mottle, curvature of petiole.	Systemic: Symptoms more mild	Systemic: Very mild mottle.
Zinnia elegans Jacq. (zinnia—var. Liliput).	Systemic: Vein clearing, yellowing, mottling, and stunting.	Systemic: More severe mottle, distortion, chlorosis.	Systemic: Same	Not tested.
Chrysanthemum morifolium Ram. (perennial chrysanthemum).	Not tested	Not tested	Systemic: Yellowing, mild mottle, stunting.	Do.

[1] Symptoms produced on the various hosts by cucumber virus 1 are designated as "type symptoms." Those produced by celery virus 1, strain 14, and strain 17 on the respective hosts are not described in detail unless they differ significantly from the "type symptoms." Symptoms are treated under 2 groups, local and systemic; when one or the other of these is omitted it is implied that they did not occur; when one or the other occurs but is similar to the "type" or the same as the "type" the fact is so stated.

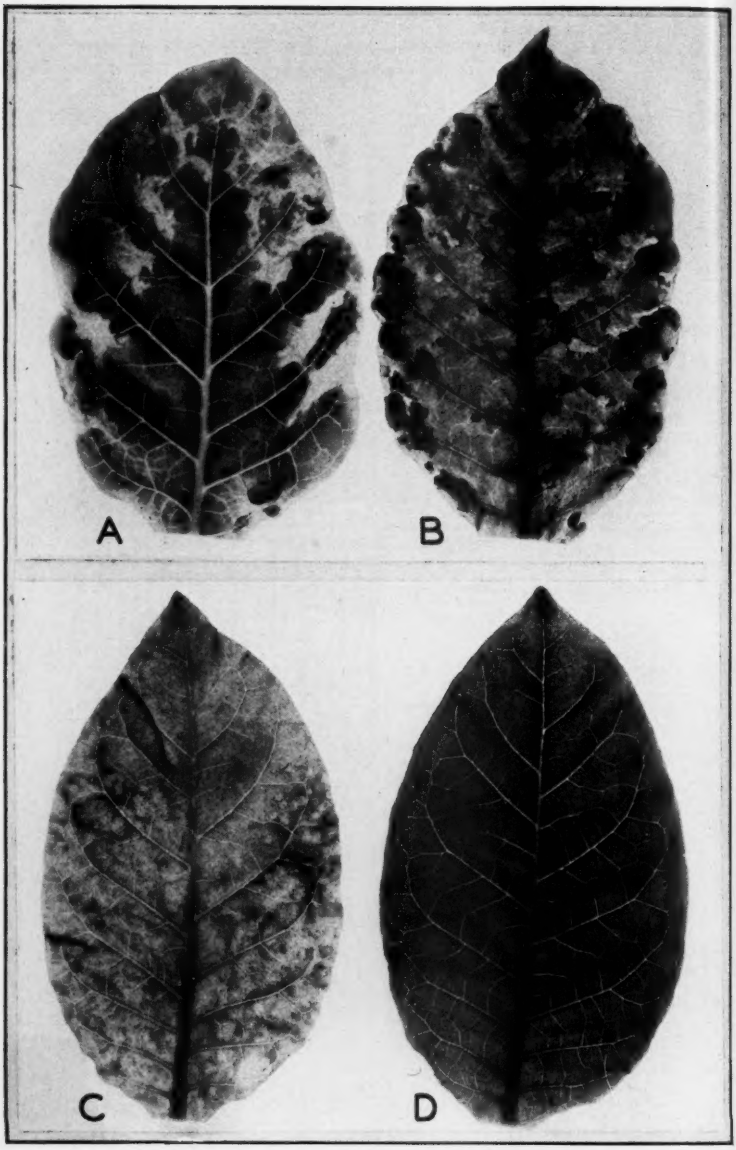

FIGURE 7.—Comparable leaves from tobacco plants inoculated with: *A*, Strain 14; *B*, cucumber virus 1; *C*, strain 17 (note the diffuse mottle); *D*, leaf from an uninoculated plant in the same series.

SPECIFIC IMMUNITY PRODUCED IN ZINNIA BY STRAIN 14

Price (*25*) has shown that zinnia plants mottled by any of several different strains of cucumber mosaic virus are immune from his strain

FIGURE 8.—*A*, Leaf from uninoculated tomato; *B*, leaf from tomato inoculated with strain 14 (note stunting and shoestring type of leaflet distortion; a diffuse mottle also occurs).

6, which produces necrotic primary lesions. Preliminary tests indicate that zinnia leaves mottled by the writers' cucumber mosaic virus strain 14 are also afforded protection from infection by strain

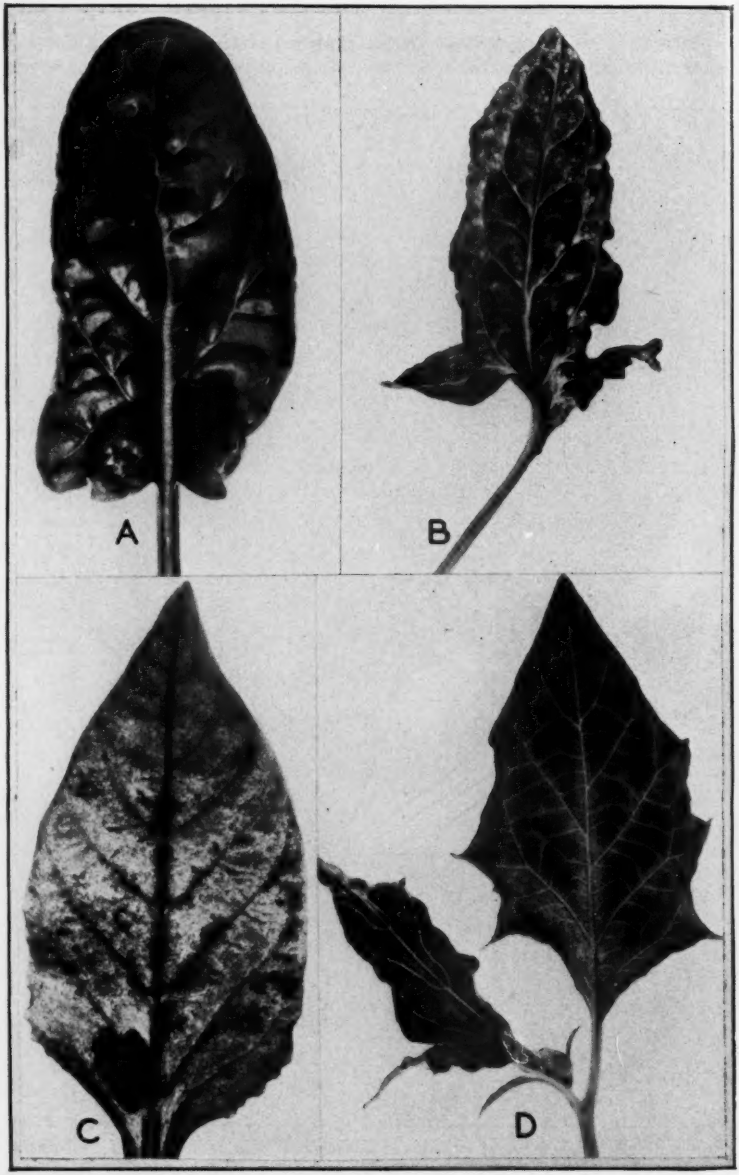

FIGURE 9.—*A*, Leaf from a healthy spinach plant; *B*, leaf from a spinach plant infected with strain 14 (note mottle, distortion, and stunting); *C*, leaf of California Wonder pepper infected with strain 14, showing mottling and dark green island; *D*, diffuse mottle and distortion in leaflets of *Datura stramonium* infected with strain 14.

6.[5] In one test 5 young zinnia plants were inoculated with strain 14 and allowed to become thoroughly mottled; then 4 leaves on each plant were inoculated with strain 6. Five healthy plants were inoculated with the latter virus only, and set apart for controls.

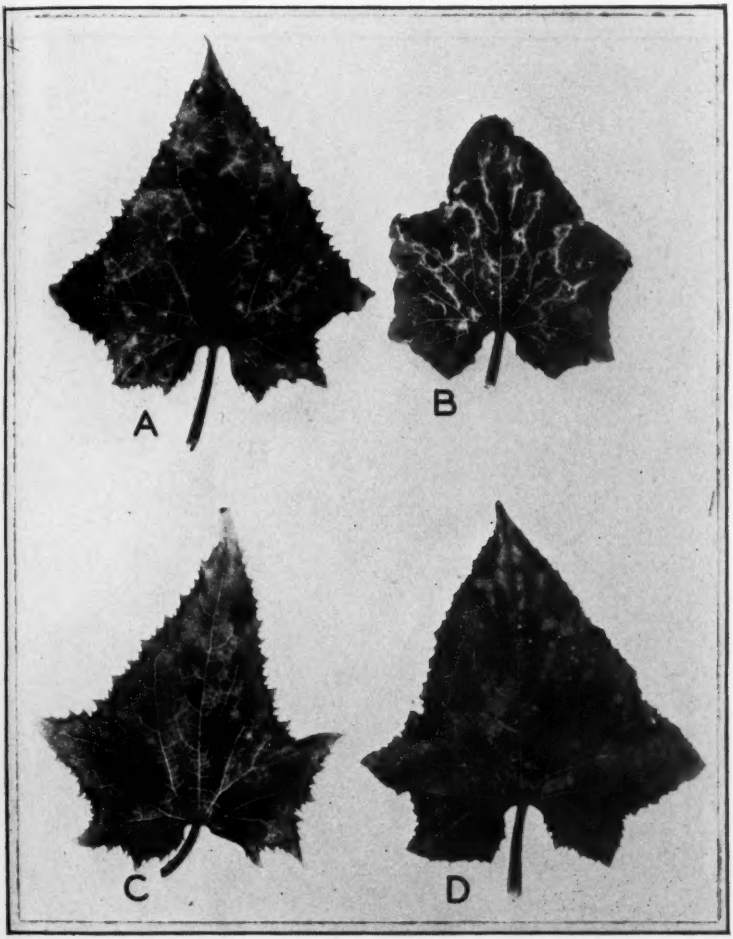

FIGURE 10.—Comparable leaves from White Spine cucumber plants inoculated with: *A*, Celery virus 1; *B*, strain 17; *C*, cucumber virus 1; *D*, strain 14 (note the similarity in the mottle symptoms on this host).

Lesions did not develop in any of the mottled leaves. The 5 control plants developed a total of 173 lesions. In a second test new leaves on each of the same 5 plants infected with strain 14 were inoculated with Price's strain 6. As in the first trial no lesions developed on the leaves mottled by strain 14, while 5 new control plants developed a

[5] A sample of cucumber mosaic virus strain 6 was kindly furnished by Dr. W. C. Price.

total of 129 lesions. A third series of 5 plants inoculated with strain 14 were again inoculated with strain 6 before all leaves showed a distinct mottle. Four lesions developed on 2 of the leaves that did not show mottling. Five control plants inoculated with strain 6 only, developed a total of 81 lesions on the same number of leaves.

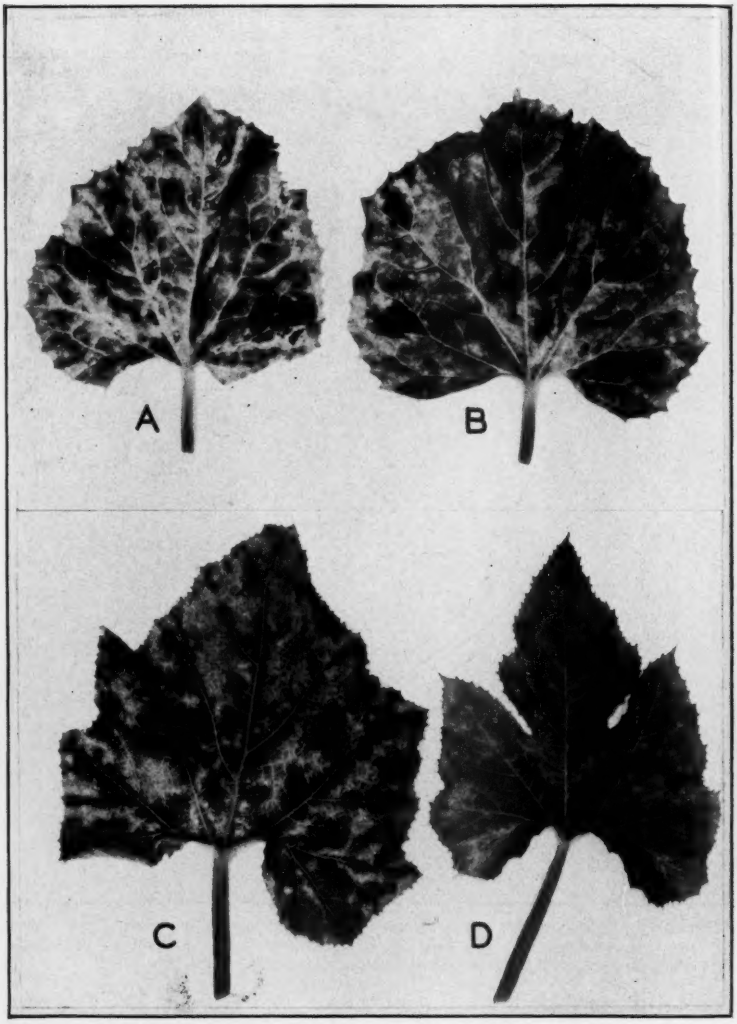

FIGURE 11.—*A*, Leaf of Honey Dew muskmelon plant inoculated with cucumber virus 1; *B*, leaf of same variety as in *A* inoculated with strain 14 (note the similarity in mottle symptoms); *C*, leaf of Cocozelle squash plant inoculated with strain 14; *D*, leaf of same variety as in *C* inoculated with strain 17 (note the more diffuse mottle in the latter).

Five zinnia plants mottled with cucumber virus 1 were also inoculated with strain 6, but no lesions developed on any of the leaves.

These preliminary tests tend to confirm Price's (*25*) report that zinnia plants mottled with cucumber mosaic virus are immune from infection by a second strain of the same virus. If the failure of

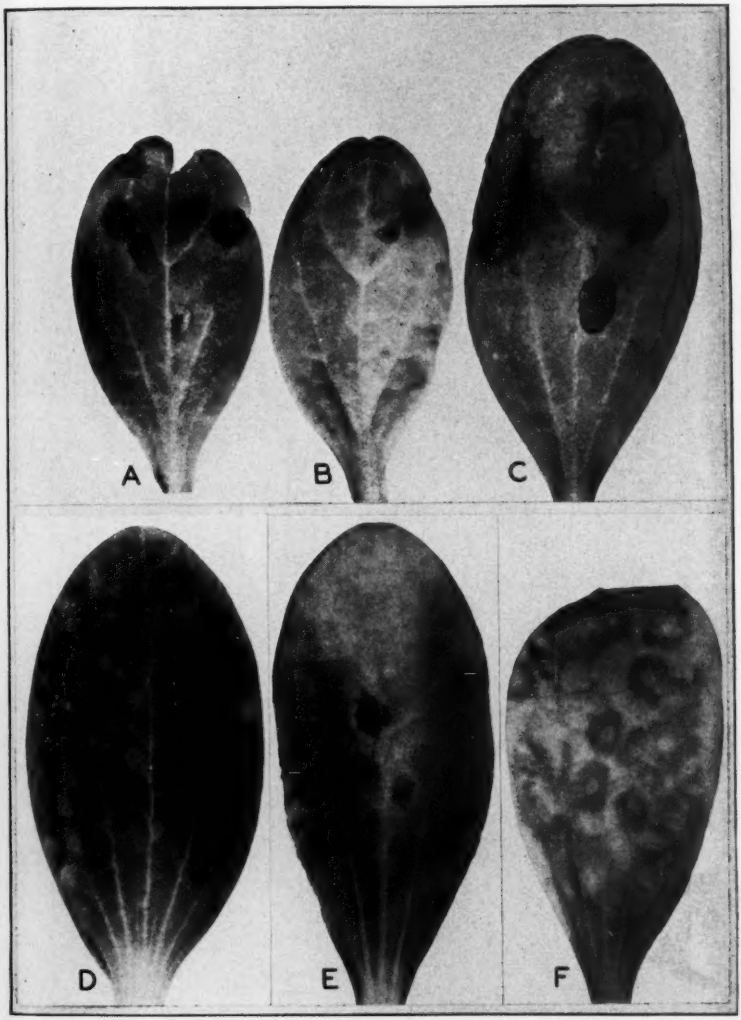

FIGURE 12.—*A*, Cotyledon of Kleckley Sweet watermelon inoculated with strain 17 (note the typical necrotic local lesions and their similarity to those on cotyledons from plants inoculated with strain 14 (*B*) and cucumber virus 1 (*C*)). *D*, *E*, *F*, Cotyledons of Cocozelle squash inoculated with strain 14: Early chlorotic lesions are shown in *D;* necrosis sometimes follows, as in *E;* commonly the early chlorotic lesions acquire a dark green halo, while the remainder of the cotyledon turns yellow, as in *F*.

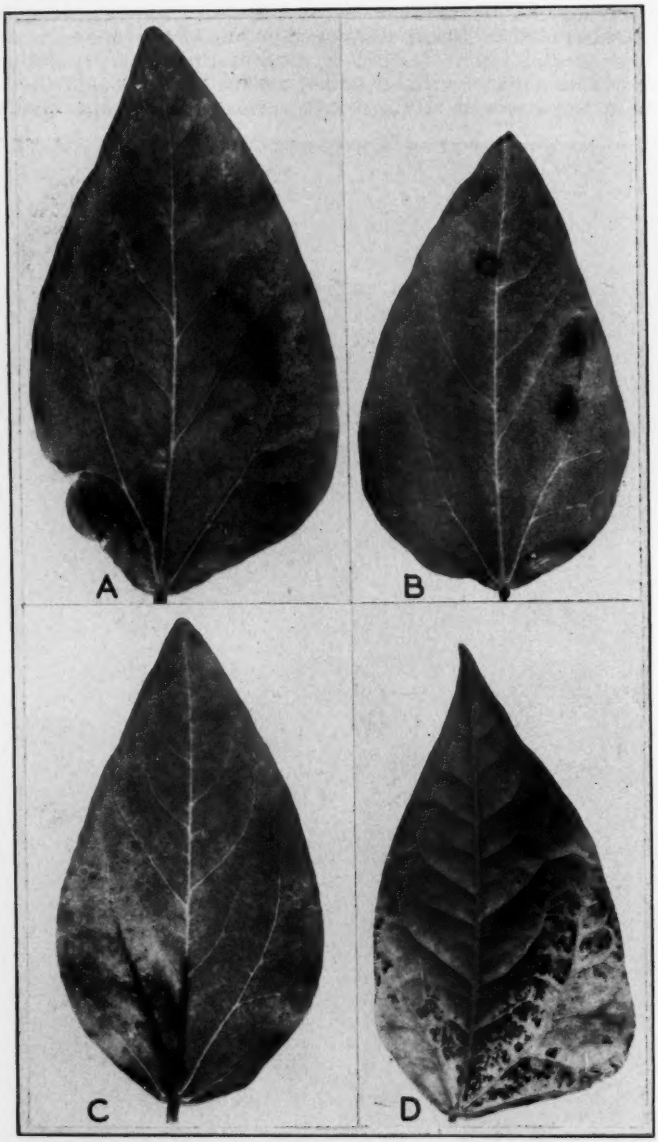

FIGURE 13.—*A*, Necrotic lesions on inoculated unifoliate leaf of Black cowpea infected with cucumber virus 1, (celery virus 1, strain 14, and strain 17 produce similar but fewer lesions on this host); *B*, larger chlorotic local lesions which have become necrotic, a type which also follows inoculation with strain 14.; *C*, later stage of type shown in *B*, necrosis having spread along the veins; *D*, bronzing and yellowing on leaflet of a first trifoliate from a Black cowpea plant infected systemically with strain 14.

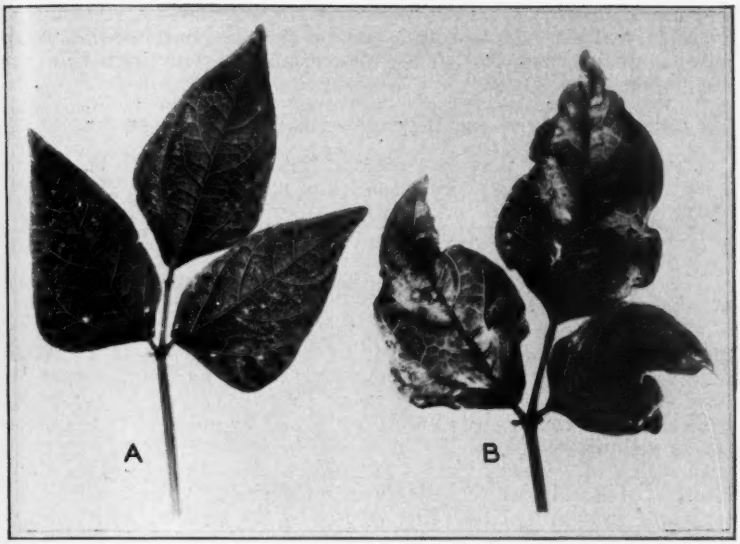

FIGURE 14.—Black cowpea: *A*, Leaf from uninoculated control; *B*, extreme mottle and distortion on leaf from plant systemically infected with strain 14.

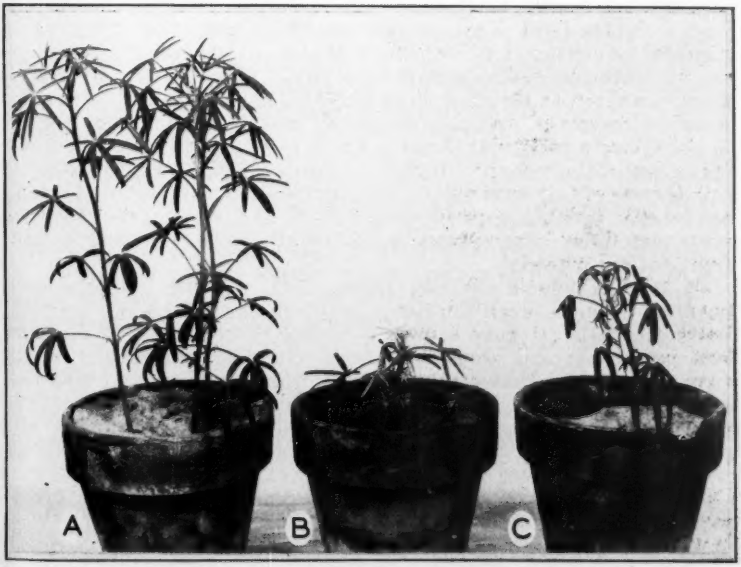

FIGURE 15.—*A*, Uninoculated plants of *Lupinus angustifolius* L.; *B*, severe stunting of plant infected with strain 14 (mottling, chlorosis, wilting, and necrosis also occur); *C*, comparable plant infected with cucumber virus 1.

necrotic lesions to develop on the leaves mottled with either cucumber virus 1 or strain 14 is due to a specific acquired immunity it would seem to demonstrate further the close relationship of strain 14 to the cucumber mosaic group.

SEPARATION FROM ONE ANOTHER OF THE FOUR VIRUSES STUDIED

Strains 14 and 17 may be separated from mixtures with bean virus 1 and bean virus 2 (*21*), pea virus 1 and pea viruses 2A, 2B, and 2C (*30*) by inoculation to either tobacco or tomato. The five last-named viruses may also be freed from strains 14 and 17 by transfer to broadbean.

The four viruses studied cannot be completely separated from one another when present in admixture. However, certain combinations of these viruses in the same plant may be readily separated out by means of differential hosts. Strain 14 can be isolated from a mixture of any of the other viruses by inoculation to kidney bean, cowpea, or Fordhook Mammoth Pod lima bean. Strain 17 can be separated from either cucumber virus 1 or celery virus 1 by inoculation to garden pea or yellow sweetclover, but it cannot be separated from a mixture with strain 14. Celery virus 1 is freed readily from the other three viruses by inoculation to broadbean. Cucumber virus 1 cannot be separated from any of the other viruses by the methods employed.

DISCUSSION

In this investigation an attempt has been made to describe and identify two viruses which were found occurring naturally on pea and bean. On the basis of symptoms, host range, modes of transmission, physical properties, and immunity studies it appears that they are related to the cucumber mosaic virus group, of which cucumber virus 1 may be given as the type virus (*4*, *15*). Since the term "cucumber mosaic virus group" has been commonly used by a number of workers in classifying a particular strain or group of related viruses, the following review of the more pertinent literature is given in an effort to orientate the use of this term and to focus attention on the fact that strains apparently isolated experimentally from the same well-recognized virus may differ in symptoms and hosts affected as do those isolated from natural material.

In 1916, Doolittle (*3*) and Jagger (*11*), working independently, reported the occurrence of a transmissible mosaic disease of cucumber. Later Doolittle (*4*) gave a more complete description of symptoms, host range, physical properties, insect and seed transmission of his virus. He found that expressed juice from mosaic plants was rendered noninfectious when heated for 10 minutes at 70° C. or aged in vitro more than 3 to 5 days, and that dilutions of 1 to 10,000 still produced infection. All species of Cucurbitaceae tested by Doolittle and Walker (*6*) were found susceptible except those of the genus *Citrullus* (watermelon and citron). Of the latter only the green-seeded citron was infected. Additional hosts in other families were found susceptible, including tobacco, pepper, *Physalis* spp., catnip (*Nepeta cataria* L.), *Martynia louisiana* Mill., milkweed (*Asclepias syriaca* L.), pokeweed (*Phytolacca decandra* L.), and pigweed (*Amaranthus retroflexus* L.). Johnson (*15*) suggested cucumber virus 1 as the

technical name for the cucumber mosaic virus and recommended that the one described by Doolittle (*4*) be regarded as the type virus.

The natural occurrence of a number of cucumber mosaic viruses which differ either in symptoms, physical properties, or host range have been reported and some have been isolated experimentally. Jagger (*12*) distinguished between "white pickle" mosaic and a second mosaic of cucumber which caused a mottle of leaves but gave rise to no symptoms on the fruit. He (*13*) also reported a third mosaic disease on summer crookneck squash and pie pumpkin which could not be transmitted to cucumber. E. M. Johnson (*14*) on the basis of symptoms produced on tobacco described the following three types of cucumber mosaic. Valleau and Johnson (*32*) had earlier characterized type 1 by the term "puffed;" this type produced symptoms on cucumber similar to those described by Doolittle (*4*). Cucumber mosaic types 2 and 3 produced different symptoms on tobacco but were similar on cucumber and certain other hosts. Porter (*22*) made a study of cucumber mosaic obtained from a number of States, including Iowa, New York, California, Kansas, and Kentucky. On the basis of symptoms produced on cucumber he concluded that all samples except one were similar to Doolittle's virus (*4*). Porter referred to these isolates as "cucumber virus 1." The isolate which differed from the others was called "cucumber virus 2"; this was obtained from Bettendorf, Iowa. In addition to differences in symptoms on cucumber (var. White Spine) the latter virus infected watermelon, West India gherkin (*Cucumis anguria* L.), and African citron (*Citrullus vulgaris* Schrad). Recently Price (*28*) has shown by immunity studies the similarity of Porter's "cucumber virus 1" and Doolittle's cucumber virus 1.

Hoggan (*10*) has described a "yellow cucumber mosaic" virus which differed from cucumber virus 1 in producing a bright yellow mottle on tobacco and other hosts, but it had identical properties, modes of transmission, and host range. It was considered a variant of cucumber virus 1. A second virus, "cucumber mild mosaic," was described by her as producing milder symptoms and being lower than cucumber virus 1 in thermal inactivation point, tolerance to dilution, and longevity in vitro. She considered that this virus also belonged to the cucumber mosaic virus group.

Price (*24*) was able to isolate a number of yellow mosaic and necrotic type viruses from the bright yellow spots which appeared on the leaves of tobacco infected with Porter's (*22*) "cucumber virus 1." Each strain was found to produce different symptoms on several hosts, but all were similar in regard to thermal death point and aging in vitro. In another experiment in which cucumber mosaic virus received from E. M. Johnson was passed from primary lesions on cowpea in successive transfers, Price obtained evidence that the virus may occasionally become altered. This occurred when certain of these transfers on cowpea developed both yellow and necrotic lesions. Subsequent transfers to cowpea from the yellow lesions again produced yellow primary lesions, a severe systemic mottle, and also a yellow mosaic in tobacco.

In England Ainsworth (*1*) has described three mosaic diseases of cucumber. Two of these, which he refers to as "green mottle mosaic" or "cucumber virus 3" and "yellow mosaic" or "cucumber virus 4," respectively, occur naturally only on cucumber and differ in host

range from the third, "yellow mottle mosaic" ("cucumber virus 1"), since they induce systemic infection of watermelon, but they are not infectious to solanaceous plants. He reports that they also differ from cucumber virus 1 in ability to resist aging in vitro for 9 months or longer, and to survive heating for 10 minutes at 80° C., though they are inactivated at 90°. The "yellow mosaic" virus differs from "green mottle mosaic" only in symptoms produced on cucumber and is regarded as a strain of the latter. Ainsworth regards cucumber viruses 3 and 4 as distinct from cucumber virus 1, the last-named being identical with Johnson's (*15*) cucumber virus 1.

It has been shown that viruses of the cucumber mosaic group are the causal agents of a number of common diseases of plants other than cucurbits. Hoggan (*9*) gave evidence that the disease originally described as "spinach-blight" by McClintock and Smith (*19*) is probably identical with cucumber mosaic (cucumber virus 1) on spinach. Wellman (*33*) has described a virus causing a serious disease of celery (celery virus 1) with a much wider host range than cucumber virus 1, but it has physical properties and symptoms similar to those of the latter virus. More recently Price (*26*) in his immunity studies has given further proof of the close relation between celery virus 1 and his strain of cucumber mosaic virus. He has also shown (*27*) that the viruses of lily mosaic, ordinary cucumber mosaic, strain 6 of cucumber mosaic, and celery mosaic produce similar symptoms on lily, and that zinnia leaves infected with the passage strain of lily mosaic virus are immune to infection from strain 6 of cucumber-mosaic virus. Wellman (*34*) has also obtained infection on banana with celery virus 1, resulting in symptoms similar to bunchy top of banana. Köhler (*18*), working in Germany, described a lupine disease which he concluded was caused by Ainsworth's (*1*) yellow mottle mosaic virus of cucumber (cucumber virus 1). Harter (*7*) obtained a virus from lima bean which he concluded belonged to the cucumber mosaic virus group.

The cucumber mosaic viruses occurring in the United States, reported above, are shown to have certain definite similarities, and those which were studied most critically gave further evidence that they belong to the same group. From the present information it appears that there are a large number of closely related viruses which may differ greatly in symptoms and hosts affected. However, cucumber virus 3 and cucumber virus 4 reported from England (*1*) appear to be distinct from cucumber virus 1 and, therefore, probably do not belong to the same group.

Strain 14, described in this paper and found occurring naturally on pea and bean, has a host range that parallels, yet exceeds, that of cucumber virus 1. In property studies the two viruses are similar, and on certain plants symptom differences are hard to distinguish. The principal difference is the ability of strain 14 to infect systemically kidney bean, Fordhook lima bean, pea, jimsonweed, cowpea, and yellow sweetclover. Cucumber virus 1 and celery virus 1 cannot be considered infectious on pea, but the fact that symptoms developed on a few plants inoculated with these viruses suggests the possibility of obtaining strains from these viruses that readily infect pea. The work of Price (*24*, *27*), who obtained a strain of cucumber mosaic systemic on cowpea and a passage strain of the lily-mosaic virus on tobacco, lends support to this hypothesis, and furnishes a plausible

explanation for the origin of strains 14 and 17. Strain 14 resembled more nearly celery virus 1 in symptoms, property studies, and host range than did any of the other viruses studied. However, there were certain hosts that were infected by strain 14 but not by celery virus 1, and vice versa.

Strain 17, also described in this paper, produces symptoms on tobacco and cucumber which are nearly identical with those of "cucumber mild mosaic virus" described by Hoggan (*10*). The properties of the two viruses do not differ significantly. Strain 17 has a host range which differs from that of cucumber virus 1 only in the ability of the former to infect pea, jimsonweed, and sweetclover systemically. Only in symptoms do the two viruses differ appreciably.

At present the natural occurrence of strains 17 and 14 has been limited to pea and bean plantings near Madison, Wis. Diseased pea plants from various parts of Wisconsin showing similar symptoms were not infected with these viruses. Whether the limited occurrence of these strains on legumes is due to their recent origin or to environmental factors is at present only a matter for speculation. Harter's (*7*) report of a cucumber mosaic virus on lima bean in Maryland and Zaumeyer's (*39*) discovery of two viruses of this group on pea in Colorado suggest a widespread occurrence of cucumber mosaic viruses on legumes. Sweetclover, which is a host of strains 14 and 17, supplies a common biennial legume in which the viruses may overwinter.

Strains 14 and 17 differ distinctly from the viruses of bean mosaic described by Pierce (*21*) and the pea viruses reported by Stubbs (*30*) in host range, properties, and symptoms on certain hosts. Zaumeyer and Wade (*40*, *41*) and more recently Zaumeyer (*38*) have reported a number of viruses affecting pea and bean. These also differ in either host range, properties, or symptoms. However, two of these (alfalfa viruses 1A and 1B) (*38*) are of special interest since they infect systemically a number of hosts susceptible to strains 14 and 17, including tobacco, zinnia, and cucumber. The properties of alfalfa-mosaic viruses 1A and 1B, including thermal inactivation point, longevity, and tolerance to dilution, are within the range reported by several workers for strains of cucumber-mosaic virus, but because of the differences in symptoms and host range it is not probable that they are related to cucumber virus 1. Nevertheless immunity studies similar to those described by Price (*25*) and other direct comparisons of the viruses might prove of value in demonstrating more distinct differences or similarities.

On the basis of the present information viruses 14 and 17 appear to be as closely related to cucumber virus 1 as a number of other viruses previously reported. Whether they should be regarded as strains or as related viruses is largely a matter of definition. In this paper they have been referred to as strains primarily for the convenience of placing them in the cucumber mosaic virus group, of which cucumber virus 1 is referred to as the type virus.

SUMMARY

The two viruses discussed in this paper, one affecting peas and the other both peas and beans, were found inducing disease on these plants in nature. Since this investigation has given evidence that the two viruses are related to cucumber-mosaic viruses they are tentatively referred to as strains 14 and 17 of that group.

The symptoms produced on peas and beans are described in some detail. Strain 14 infected all 25 strains of bean and the 3 varieties of pea tested. Minor varietal and strain differences were observed and examples of these are given. Strain 17 infected the 3 varieties of peas tested but none of the 25 strains of bean. Neither cucumber virus 1 nor celery virus 1 infected any of the above varieties of bean systemically, but a few pea plants inoculated with these viruses developed local symptoms. However, infection could not be obtained in successive experiments, and for this reason peas are not regarded as hosts of the last-named viruses.

Three substrains of 14 were isolated; two of these remained stable and produced distinct symptoms on tobacco, cucumber, bean, pea, and cowpea. The "yellow" isolate produced a more severe disease while the "dark green" isolate was less virulent than the original strain.

On plants infected with strain 14 the disease was most severe at temperatures of 24° to 28° C., within which range stunting, mottling, and necrosis occurred. Inoculated plants held at 16° developed no symptoms except stunting and epinasty of inoculated primary bean leaves.

The properties of strains 14 and 17 were found to agree in general with those described for several cucumber-mosaic viruses. Strains 14 and 17 remained infectious in vitro 7 days at 20° to 22° C. The tolerance to dilution is about 1 to 10,000 for strain 14 and 1 to 1,000 for strain 17. The thermal inactivation point for strain 14 is about 65° C. and for strain 17 between 65° and 70°.

The two viruses were readily transmitted by means of the peach aphid to and from a number of host plants. Mechanical transmission was also accomplished with or without the use of carborundum powder as an abrasive. No seed transmission was obtained in 580 pea and 440 bean seedlings grown from seed of plants infected with strain 14. Seed transmission was not studied with strain 17.

Table 3 gives a summary of the hosts infected and cardinal symptoms produced by the 4 viruses. This list includes 19 species belonging to 16 genera and 6 families. Strains 14 and 17 infected all plants susceptible to cucumber virus 1, and strain 14 was pathogenic on a number of additional hosts. Strain 17 differed from cucumber virus 1 only in systemic infection on pea, jimsonweed, and yellow sweetclover. The host range of strain 14 closely paralleled that of celery virus 1 on the plants tested, but each virus infected certain hosts not susceptible to the other. On some hosts the symptoms of strains 14 and 17 were similar to those of cucumber virus 1, but on other hosts certain differences were evident. Soybean (var. Manchu) and White Dutch clover were not infected by any of the 4 viruses.

Preliminary immunity studies give further indication of the close relationship of strain 14 to the cucumber mosaic group.

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TULIP ANTHRACNOSE [1]

By C. M. TOMPKINS, *assistant plant pathologist*, and H. N. HANSEN, *associate plant pathologist, California Agricultural Experiment Station*

INTRODUCTION

An anthracnose disease, affecting primarily the peduncles and leaf blades of Darwin tulips (*Tulipa gesneriana* L.) was found in a garden at Burlingame, Calif., in April 1939. A preliminary report [2] of the disease has already been given. The symptoms of the disease and the identity of the causal organism are discussed in this paper.

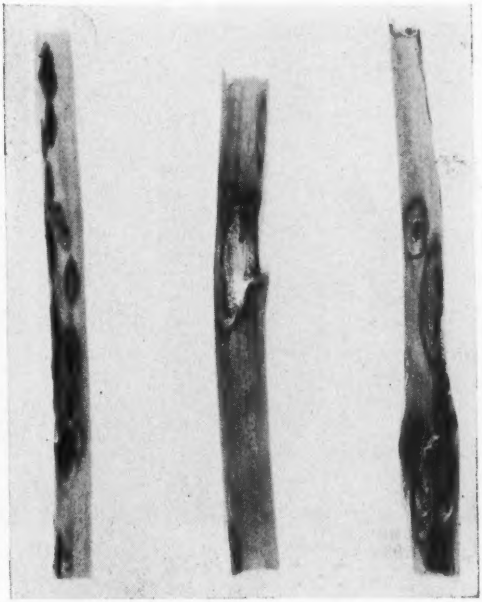

FIGURE 1.—Natural infection of tulip peduncles, showing elliptical lesions containing small, black acervuli. Collected at Burlingame, Calif.

SYMPTOMS OF THE DISEASE

Symptoms of the disease on Rev. H. Eubank and Zwanenburg, the only varieties of the tulip known to be susceptible to natural infection, were identical in all respects. They consisted of small to large elliptical lesions, 0.5 to 2 cm. in length and 0.2 to 1 cm. in width, on both peduncles (fig. 1) and leaf blades. In general, the long axes of the lesions were parallel to the long axes of peduncles and leaf blades. In the early stages of infection, the lesions are water-soaked; later, they become dry and black around the margins. Numerous small, black acervuli develop in the central part of the lesions.

THE PATHOGEN

The causal fungus was readily isolated on potato-dextrose agar either by tissue plantings from peduncles or leaf blades, or by direct

[1] Received for publication August 14, 1940. Contribution from the Division of Plant Pathology, California Agricultural Experiment Station.

[2] TOMPKINS, C. M., and HANSEN, H. N. TULIP ANTHRACNOSE. (Abstract) Phytopathology 30: 790. 1940.

transfer of spores from the lesions. In either case, the fungus sporulates very sparsely. Only a few tiny acervuli are produced on the surface of the medium, and they are entirely covered by a profuse growth of aerial mycelium. Like many other imperfect fungi, this fungus appears to occur in nature in the dual condition,[3] for, when 50 single-spore cultures were made, 3 culture types appeared, 1 producing acervuli and conidia in great abundance with little or no aerial mycelium (fig. 2, *B*), 1 producing few conidia and abundant mycelium (fig. 2, *C*), and 1 like the parent (fig. 2, *A*).

Young tulip plants, varieties Rev. H. Eubank and Zwanenburg, were placed in a moist chamber for 24 hours and then inoculated by

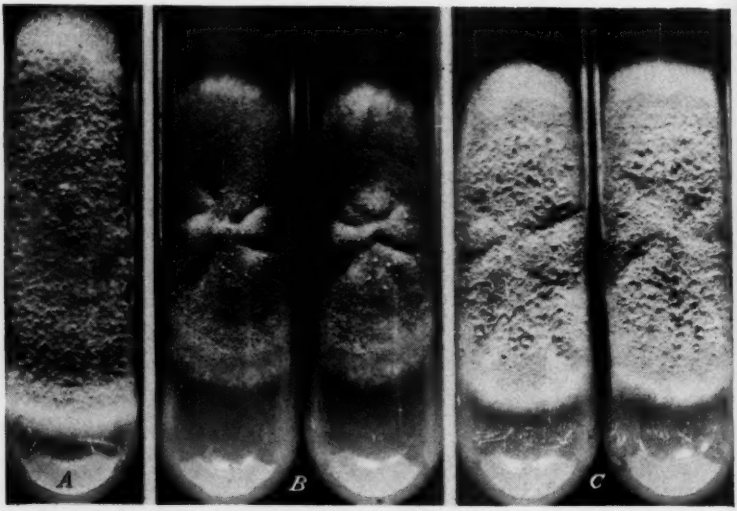

FIGURE 2.—Three culture types of the pathogen: *A*, The primary isolate; *B*, the conidial type; *C*, the mycelial type derived by single-sporing from *A*.

atomizing with a heavy suspension of spores in water. The inoculated plants were then returned to the moist chamber for an additional 24 hours, after which they were placed on a bench in the greenhouse. Control plants were not atomized. Infection, as indicated by typical lesions on peduncles and leaf blades (fig. 3, *A*, *B*), was obtained in 7 to 10 days. Under greenhouse conditions, the flowers were also infected. Additional, successful inoculations were made by atomizing the plants directly on benches in the greenhouse, without using a moist chamber. In all cases, the symptoms produced by artificial inoculation were identical with those produced in nature. The control plants remained healthy. The fungus was reisolated, and the reisolates proved pathogenic by inoculation. No infection was obtained by inoculation of the variety Clara Butt which, together with Fantasy, appears to be immune.

It was observed that the first two culture types (fig. 2, *B*, *C*) upon single-sporing remained constant, whereas the parent type (fig. 2, *A*)

[3] HANSEN, H. N. THE DUAL PHENOMENON IN IMPERFECT FUNGI. Mycologia 30: 442-455, illus. 1938.

again gave rise to the three types. On tulip, the fungus is a typical *Gloeosporium*, producing conidia in well-defined acervuli from 0.2 to 1.5 mm. in diameter. The conidia vary greatly in size from 9.75μ to 23.75μ by 3.25μ to 7.25μ, with the greater number measuring about 15.0μ by 5.0μ.

In reading descriptions of the many species of *Gloeosporium* in Saccardo,[4] one cannot fail to notice how closely many of the species

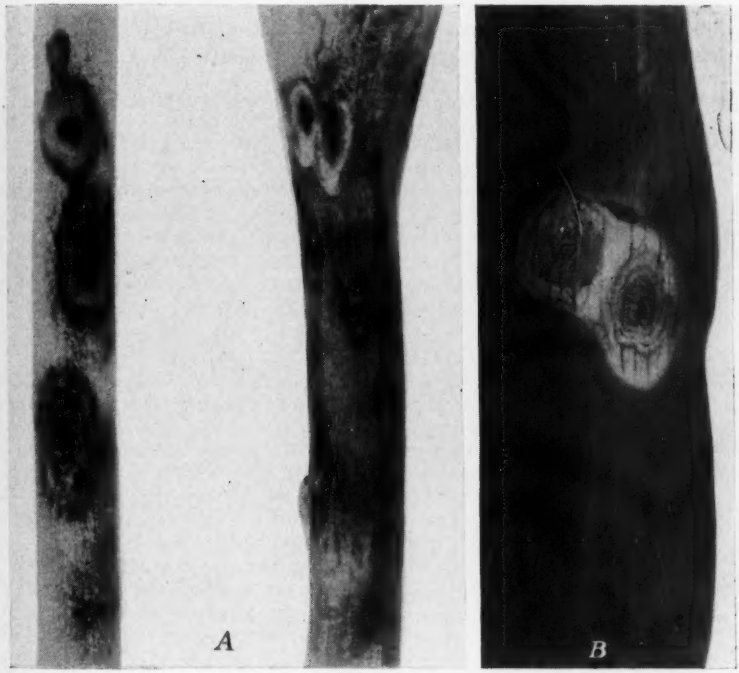

FIGURE 3.—Artificial infection of Zwanenburg tulips produced by atomizing with a water suspension of spores in the greenhouse: *A*, Infected peduncles; *B*, infected leaf blade. All lesions show a water-soaked margin, while the central areas contain numerous black acervuli.

resemble each other morphologically. Seemingly, habitat or host has in most instances served as the main basis for separation. Recognizing the inadequacy of such a system, it seems desirable to refrain from further encumbering the literature by adding a new species to a genus already so numerously represented. The lead recently set in the taxonomic treatment of fusaria [5] will be followed. Thus, this fungus is considered to be a form of the oldest species of *Gloeosporium* occurring on a monocot, the description of which will fit within the limits of measurements given above. The name *Gloeosporium thumenii* Sacc. f. *tulipae* forma nov. is proposed.

The method of overwintering of the fungus has not been determined.

[4] SACCARDO, P. A. SYLLOGE FUNGORUM OMNIUM HUCUSQUE COGNITORUM. v. 3, p. 721. 1884.
[5] SNYDER, W. C., and HANSEN, H. N. THE SPECIES CONCEPT IN FUSARIUM. Amer. Jour. Bot. 27: 64–67. 1940.

SUMMARY

Tulip anthracnose occurs at Burlingame, Calif.

Symptoms consist of elliptical lesions which at first are water-soaked, but later become dry and covered with fruiting bodies.

Infection of the varieties Rev. H. Eubank and Zwanenburg was obtained in 7 to 10 days by atomizing the plants with a water suspension of spores. The varieties Clara Butt and Fantasy appear to be immune in the field and in the greenhouse.

The name *Gloeosporium thumenii* Sacc. f. *tulipae* forma nov. is proposed.

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